(54) Title: BCL-G POLYPEPTIDES, ENCODING NUCLEIC ACIDS AND METHODS OF USE

(57) Abstract: The invention provides Bcl-G polypeptides and encoding nucleic acids. Bcl-G polypeptides include Bcl-G$_L$ and Bcl-G$_S$. The invention also provides mouse Bcl-G. The invention also provides vectors containing Bcl-G nucleic acids, host cells containing such vectors, Bcl-G anti-sense nucleic acids and related compositions. The invention additionally provides Bcl-G oligonucleotides that can be used to hybridize to or amplify a BcL-G nucleic acid. Anti-Bcl-G specific antibodies are also provided. Further provided are kits containing Bcl-G nucleic acids or Bcl-G specific antibodies. Such kits and reagents can be used to diagnose cancer, monitor response to therapy, or predict the prognosis of a cancer patient. The invention additionally provides methods of modulating apoptosis using Bcl-G polypeptides, encoding nucleic acids, or compounds that modulate the activity or expression of Bcl-G polypeptides. The methods for modulating apoptosis can be used to treat diseases such as cancer.

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BCL-G POLYPEPTIDES, ENCODING NUCLEIC ACIDS AND METHODS OF USE

BACKGROUND OF THE INVENTION

The present invention relates generally to regulation of programmed cell death and more specifically to molecules that promote programmed cell death.

In essentially all self-renewing tissues, a balance is struck between cell production by mitogenesis and cell loss due to programmed cell death, thereby maintaining total cell numbers within a physiologically appropriate range. In pathological conditions, however, the balance in cell production and cell loss can be disrupted. In cancer, for example, an increased amount of cell production due to a shortened cell cycle time or a decreased amount of cell death due to dysregulation of a programmed cell death pathway results in the growth of a tumor.

With regard to programmed cell death, a variety of stimuli, which occur either external or internal to the cell, initiate a pathway that ultimately results in apoptosis of the cell. As is common for most signal transduction pathways, the various different stimuli that induce apoptosis likely initiate the process of programmed cell death through specific pathways. However, most if not all of these initial pathways converge at a common point that generally involves a

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member of the Bcl-2 family of proteins.

The Bcl-2 family of proteins regulate a distal
step in the evolutionarily conserved pathway for
programmed cell death and apoptosis, with some members of
5   this family functioning as suppressors of cell death
(anti-apoptotic proteins) and other members functioning
as promoters of cell death (pro-apoptotic proteins).
Overexpression of the anti-apoptotic protein, Bcl-2, for
example, blocks neuronal cell death that otherwise is
10   induced *in vitro* by various stimuli, including
neurotrophic factor withdrawal, various oxidants, glucose
deprivation, certain neurotrophic viruses, and amyloid
β-peptide.   In addition, Bcl-2 is overexpressed in some
tumor cells and, in part, may contribute to tumor growth
15   by altering the balance between cell division and cell
death.

The Bcl-2 family of proteins are critical
regulators of pathways involved in apoptosis, acting to
either inhibit or promote cell death (Reed, Nature
20   387:773-776 (1997); Green and Reed, Science 281:1309-1312
(1998); Reed, Oncogene 17:3225-3236 (1998); Reed, Curr.
Opin. Oncol. 11:68-75 (1999)).   The Bcl-2 family members
can be divided into two groups, those with anti-apoptotic
activity, including Bcl-2 and Bcl-X$_L$, and those with pro-
25   apoptotic activity, including Bax and Bak.

Four distinct domains have been identified in
Bcl-2 family members, designated BH1 to BH4.   The BH4
domain is a domain that mediates interactions with a
variety of cellular proteins (Reed, *supra*, 1998).   The
30   BH1, BH2 and BH3 domains form a binding pocket for
dimerization with other Bcl-2 members having a BH3
domain, which also functions as a ligand that binds to

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the dimerization binding pocket. The dimerization
function of the Bcl-2 members is an important mechanism
for regulating apoptosis in that heterodimerization of
pro-apoptotic Bcl-2 members with anti-apoptotic Bcl-2
5   members can regulate the cellular apoptotic pathways.
Some Bcl-2 members only have a BH3 domain and therefore
function as trans-dominant inhibitors of anti-apopototic
proteins such as Bcl-2 and Bcl-X_L (Reed, *supra*, 1998).

Another function of Bcl-2 members is the
10   formation of ion channels. Bcl-2 members can localize to
the mitochondrial membrane, and the formation of ion
pores that alter the permeability of mitochondria is
thought to be an important signaling mechanism for the
induction of apoptosis. Thus, Bcl-2 members use at least
15   three mechanisms to regulate apoptotic activity:
dimerization with Bcl-2 members, formation of ion pores
in mitochondria, and binding to non-Bcl-2 members that
function as signaling molecules.

In comparison, overexpression of the
20   pro-apoptotic protein, Bax, for example, promotes cell
death when triggered by a variety of inducers of
apoptosis, including growth factor withdrawal, ionizing
radiation, and anti-Fas antibody. In addition,
elevations in Bax expression occur in association with
25   cell death induced by a variety of stimuli, including
neuronal cell death that occurs due to ischemia,
epilepsy, spinal cord injury, and certain
neurodegenerative diseases such as Parkinson's disease
and Alzheimer's disease.

30   Although aberrant expression of members of the
Bcl-2 family of proteins is associated with various
pathologic conditions, the mechanisms by which these

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proteins mediate their action is not known. Often, the
action of a protein can be inferred from its structural
relationship to other proteins, whose functions are
known. However, while the Bcl-2 family proteins share
5   certain structural homologies with each other, they do
not share substantial amino acid sequence homology with
other proteins, further hindering attempts to understand
how the Bcl-2 family proteins such as Bcl-2 and Bax
regulate cell death.

10   Thus, a need exists to identify proteins
involved in the programmed cell death pathway and to
identify methods of regulating programmed cell death for
therapeutic applications, including treatment of cancer.
The present invention satisfies this need and provides
15   related advantages as well.

SUMMARY OF THE INVENTION

In accordance with the present invention, there
are provided Bcl-G polypeptides and encoding nucleic acid
molecules. The invention polypeptides and encoding
20   nucleic acids are useful for modulating apoptosis by
altering the expression or activity of Bcl-G. The Bcl-G
polypeptides and encoding nucleic acids can be
advantageously used to diagnose or treat cancer, in
particular prostate, ovarian and leukemia. Furthermore,
25   the Bcl-G polypeptides and encoding nucleic acids are
useful to generate or screen for agents that can alter
Bcl-G activity or expression, which can further be used
to treat cancer. Bcl-G polypeptides include Bcl-G_L and
Bcl-G_S.

30   The invention also provides vectors containing
Bcl-G nucleic acids, host cells containing such vectors,

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Bcl-G anti-sense nucleic acids and related compositions.
The invention additionally provides Bcl-G
oligonucleotides that can be used to hybridize to or
amplify a Bcl-G nucleic acid. Anti-Bcl-G specific
5   antibodies are also provided. Further provided are kits
containing Bcl-G nucleic acids or Bcl-G specific
antibodies. Such kits and reagents can be used to
diagnose cancer, monitor response to therapy, or predict
the prognosis of a cancer patient. The invention
10   additionally provides methods of modulating apoptosis
using Bcl-G polypeptides, encoding nucleic acids, or
compounds that modulate the activity or expression of
Bcl-G polypeptides. The methods for modulating apoptosis
can be used to treat diseases such as cancer.

15   **BRIEF DESCRIPTION OF THE DRAWINGS**

            Figure 1 shows the nucleotide sequence of human
Bcl-G_L cDNA (SEQ ID NO:1).

            Figure 2 shows the nucleotide sequence of the
coding region of human Bcl-G_L cDNA (nucleotide 196-1179 of
20   SEQ ID NO:1) and the encoded amino acid sequence (SEQ ID
NO:2). Bcl-G_L contains a BH3 domain (^{216}LKYSGDQLE^{224}; SEQ
ID NO:5) and a BH2 domain (^{307}PWIQQHGGWE^{316}; SEQ ID NO:6).

            Figure 3 shows the nucleotide sequence of human
Bcl-G_S cDNA (SEQ ID NO:3).

25   Figure 4 shows the nucleotide sequence of the
coding region of human Bcl-G_S cDNA (nucleotide 196-954 of
SEQ ID NO:3) and the encoded amino acid sequence (SEQ ID
NO:4). Bcl-G_S contains only the BH3 domain
(^{216}LKYSGDQLE^{224}; SEQ ID NO:5).

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Figure 5 shows sequence analysis of Bcl-G
cDNAs. Figure 5A shows the predicted amino acid
sequences of the Bcl-G$_L$ and Bcl-G$_s$ proteins, with the BH2
and BH3 domains in bold-type and residue numbers
5   indicated. The predicted proteins are identical from
residues 1-226. The unique C-terminal region of Bcl-G$_s$ is
indicated in italics type. Figure 5B shows an alignment
of the BH2 domains of Bcl-G$_L$ (SEQ ID NO:9) and several
other Bcl-2 family proteins (SEQ ID NOS:10-17,
10   respectively). Identical and similar residues are shown
in black and gray blocks, respectively. Figure 5C shows
an alignment of the BH3 domains Bcl-G (SEQ ID NO:18) and
several other Bcl-2 family proteins (SEQ ID NOS:19-26,
respectively). Figure 5D shows the exon-intron
15   organization of the BCL-G gene. The BCL-G gene contains
6 exons, spanning a ~30 kb region of chromosome 12.
Alternative splicing at the 5'-end of exon 5 accounts for
the production of the Bcl-G$_L$ and Bcl-G$_s$ proteins, where
splice-acceptor sites at nucleotide positions 63,870
20   versus 63,797 in BAC clone RPCI 11-267J23 (GenBank
AC007537) are utilized for Bcl-G$_L$ and Bcl-G$_s$,
respectively. The positions of the start and termination
codons are indicated, with coding regions in gray blocks
and non-coding 5'-UTR and 3'-UTR sequence in open blocks.
25   The BH3 domain is located in exon 4 of both Bcl-G$_L$ and
Bcl-G$_s$, while the BH2 domain resides in exon 5 of Bcl-G$_L$.

Figure 6 shows mapping of Bcl-G to chromosome
12p12.3.

Figure 7 shows expression of Bcl-G$_s$ and Bcl-G$_L$
30   in human tissues. The expression of transcripts encoding
Bcl-G$_L$ or Bcl-G$_s$ was examined by RT-PCR. First-strand
cDNA prepared using RNA samples from various adult human
tissues was PCR amplified using primers specific for Bcl-

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G_L and Bcl-G_s, based on differences in splice-acceptor utilization in exon 5. The primers flank an intron in both cases, thus excluding amplification due to contaminating genomic DNA. PCR products were size-

5   fractionated in 2% agarose gels, stained with ethidium bromide, then photographed under UV-illumination.

     Figure 8 shows the effect of Bcl-G_s on cell death. PC-3 cells were transfected with pcDNA3.1/Myc/His (control), pcDNA3.1/Myc/His/Bcl-G_s (Bcl-G_s),

10   pcDNA3.1/Myc/His/Bcl-G_s + pRC/CMV/Bcl-2 (Bcl-G_s + Bcl-2), pRC/CMV/Bax (Bax), or pRC/CMV/Bax + pRC/CMV/Bcl-2 (Bax + Bcl-2). Cells were tested for cell death 24 hours after transfection.

     Figure 9 shows induction of apoptosis by Bcl-G.

15   Figure 9A shows the results of transfecting plasmids encoding GFP, GFP-Bcl-G_s, or GFP-Bcl-G_L into Cos-7 cells alone or in combination with a plasmid encoding Bcl-X_L. Apoptosis was examined by DAPI staining at 24 h post-transfection (mean + SD; n = 3) (top). Levels of GFP and

20   GFP-Bcl-G fusion proteins were examined by immunoblotting lysates from transfected Cos-7 cells (20 μg per lane) and anti-GFP antibody with ECL-based detection (middle). Equal loading was confirmed by reprobing the same membrane with anti-Tubulin antibody (bottom). Figure 9B

25   shows the results of transfecting plasmids encoding GFP, GFP-Bcl-G_s or the mutant proteins, Bcl-G_s (ΔBH3) and GFP-Bcl-G_s (L216E) into Cos-7 cells. The percentage of apoptotic cells was examined 1 day later as above (top). Protein expression was assessed by immunoblotting as

30   above, using anti-GFP (middle) or anti-Tubulin (bottom) antibodies.

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Figure 10 shows interactions of Bcl-G$_S$ and Bcl-G$_L$ with Bcl-X$_L$. 293T cells were transiently transfected with plasmids encoding GFP, GFP-Bcl-G$_L$, GFP-Bcl-G$_S$, GFP-Bcl-G$_S$ (DBH3), or GFP-Bcl-G$_S$ (L218E). Cells were lysed 1 day later and immunoprecipitations were performed using anti-GFP antibody. Immune-complexes (prepared from 2 mg lysate) (top) and lysates (20 μg protein) (bottom) were subjected to SDS-PAGE/immunoblot analysis using anti-Bcl-X$_L$ (top) and anti-GFP (bottom) antibodies, respectively.

Figure 11 shows microscopic evaluation of intracellular distributions of Bcl-G$_L$ and Bcl-Gs. Plasmids encoding GFP (A), GFP-Bcl-G$_L$ (B), GFP-Bcl-G$_S$ (C), and GFP-Bcl-G$_S$ (ΔBH3) (D) were transfected into Cos-7 cells. Cells were fixed 1 day later and examined by confocal microscopy.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided nucleic acids encoding Bcl-G polypeptides, or functional polypeptide fragments thereof. As used herein, the term "Bcl-G" refers to sub-family members of the Bcl-2 family of proteins, wherein said Bcl-G comprises a BH3 domain (SEQ ID NOS:5 or 9). The human Bcl-G gene has been found to map to chromosome 12p12.3 (Example II). ·This region of chromosome 12 is frequently deleted in cancer cells, in particular in acute lymphoblastic leukemia (ALL) and other solid tumor cells (Baens et al., (1999) Genomics 56:40-50 (1999); Hatta et al., Br. J. Cancer 75:1256-1262 (1997); Kibel et al., Cancer Res. 58:5652-5655 (1998); Baccichet et al., Br. J. Haematol. 99:107-114 (1997); Aissani et al., Leuk. Lymphoma 34:231-239). This region is deleted in a subset of prostate (approximately 50%), ovarian (approximately

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30%) and leukemias (approximately 30%). Therefore, Bcl-G
can function as a tumor suppressor. Furthermore, the
presence or absence of Bcl-G nucleic acid or polypeptide
or changes in Bcl-G nucleic acid or polypeptide
5   expression, can serve as a marker for predisposition or
progression of cancer, for example, prostate, ovarian and
leukemia. Thus, the invention Bcl-G nucleic acids and/or
polypeptides can be used for screening for cancer and/or
for developing drug candidates for the treatment of
10   cancer. Invention Bcl-G nucleic acids and/or
polypeptides can also be used for discovery of drugs, as
disclosed herein, that suppress autoimmunity,
inflammation, allergy, allograph rejection, sepsis, and
other diseases, including inflammatory diseases.

15   A new member of the Bcl-2 family was
identified, Bcl-G (see Examples). The human *BCL-G* gene
consists of 6 exons, resides on chromosome 12p12, and
encodes two proteins through alternative mRNA splicing:
Bcl-G (long) and Bcl-G (short) consisting of 327 and 252
20   (length) amino acids, respectively. $Bcl-G_L$ and $Bcl-G_S$ are
identical in their first 226 amino-acids but diverge
thereafter. Among the Bcl-2 Homology (BH) domains
previously recognized in Bcl-2 family proteins, the BH3
domain is found in both $Bcl-G_L$ and $Bcl-G_S$, but only the
25   longer $Bcl-G_L$ protein possesses a BH2 domain. $Bcl-G_L$ mRNA
is expressed widely in normal human tissues, whereas Bcl-
G_S mRNA was found only in testis. Over-expression of Bcl-
G_L or $Bcl-G_S$ in cells induced apoptosis, but $Bcl-G_S$ was
far more potent than $Bcl-G_L$. Apoptosis induction by Bcl-
30   GS depended on the BH3 domain, and was suppressed by co-
expression of anti-apoptotic Bcl-XL protein. Bcl-XL also
co-immunoprecipitated with $Bcl-G_S$ but not with mutants of
$Bcl-G_S$ in which the BH3 domain was deleted or mutated and
not with $Bcl-G_L$. $Bcl-G_S$ was predominantly localized to

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cytosolic organelles whereas Bcl-G$_L$ was diffusely
distributed throughout the cytosol. The findings suggest
that Bcl-G$_L$ is likely in a latent state, whereas the
shorter Bcl-G$_S$ protein is constitutively active.

5   The term "biologically active" or "functional",
when used herein as a modifier of an invention Bcl-G, or
polypeptide fragment thereof, refers to a polypeptide
that exhibits functional characteristics similar to Bcl-
G, including those disclosed herein (see Examples I-IX).
10   As disclosed herein, Bcl-G induces apoptosis (see Example
IV). Therefore, one function of Bcl-G is a pro-apoptotic
function. The pro-apoptotic function of Bcl-G is
inhibited by co-expression of the anti-apoptotic protein
Bcl-2 (Example IV). Therefore, another function of Bcl-G
15   is modulation by or interaction with an anti-apoptotic
protein such as for example, Bcl-2 family member,
including Bcl-2 or Bcl-X$_L$, and the like. Bcl-G can
function to heterodimerize with a Bcl-2 family member,
thereby modulating the apoptotic activity of Bcl-G and/or
20   the Bcl-2 family member. For example, the interaction of
BC1-G$_S$ with Bcl-X$_L$ was found to be BH3 domain dependent,
and, thus, the pro-apoptotic activity of Bcl-G$_S$ correlates
with its ability to bind Bcl-X$_L$ (see Example VI).

        Bcl-G is also contemplated herein as having the
25   ability to function as an ion channel. Additionally,
Bcl-G is contemplated herein as having the ability to
function target to mitochondria, for example, for
example, by binding directly to mitochondria or via
binding to a protein that is associated with mitochondria
30   such as Bcl-2 or Bcl-X$_L$. Bcl-G can also function to bind
adenine nucleotide transporter (ANT) and to other
proteins such as voltage-dependent anion channel (VDAC).

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Because Bcl-G is located on chromosome 12 in a region that is frequently deleted in cancer cells (Example II) it is contemplated herein that Bcl-G functions as a tumor suppressor. Another functional activity of Bcl-G is the ability to act as an immunogen for the production of polyclonal and monoclonal antibodies that bind specifically to an invention Bcl-G. Thus, an invention nucleic acid encoding Bcl-G will encode a polypeptide specifically recognized by an antibody that also specifically recognizes the Bcl-G protein including the amino acid sequence, set forth in SEQ ID NOS:2, 4 or 42. Such immunologic activity can be assayed by any method known to those of skill in the art. Therefore, Bcl-G functional fragments include polypeptide fragments that function as immunogens for generating a Bcl-G-specific antibody and fragments that specifically bind to a Bcl-G-specific antibody.

Bcl-2 family proteins are central regulators of apoptosis (reviewed in Reed, J. C., _Nature_, 387:773-776 (1997); Adams & Cory, _Science_, 281:1322-1326 (1998); Gross et al., _Genes Dev._, 13:1899-1911 (1999)). Bcl-2 family proteins are conserved throughout the animal kingdom, with homologues identified in both vertebrates and invertebrates. These proteins contain up to four conserved Bcl-2 Homology (BH) domains, BH1, BH2, BH3, and BH4, which are recognized by their amino-acid sequence similarity. Both anti-apoptotic and pro-apoptotic Bcl-2 family proteins have been identified. These proteins control cell life-death decisions through their effects on events such as mitochondrial release of proteins involved in activation of caspase-family cell death proteases (reviewed in Gross et al., _Genes Dev._, 13:1899-1911 (1999); Green & Reed, _Science_, 281:1309-1312 (1998); Kroemer & Reed, _Nature Medicine_, 6:513-519 (2000)). Many

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Bcl-2 family proteins are capable of physically
interacting with each other, forming a complex network of
homo-and heterodimers, and these physical interactions
sometimes play important roles in the opposing effects of
5   pro- and anti-apoptotic members of the family.

        The pro-apoptotic members of the Bcl-2 family
can be broadly classified into two groups. One group,
including Bax, Bak, and Bok in humans, shares structural
similarity with the pore-forming domains of certain
10   bacterial toxins and is capable of forming pores in
synthetic membranes *in vitro* (Schendel et al., Cell Death
Differ., 5:372-380 (1998); Antonsson et al., Science,
277:370-372 (1997); Schlesinger et al., Proc. Natl. Acad.
Sci. USA, 94:11357-11362 (1997); Shimizu et al., J Biol
15   Chem., 16:12321-12325 (2000)). These protein exhibit
cytotoxic effects independently of their ability to bind
other Bcl-2 family proteins, including Bcl-2 and other
cytoprotective members of the family such as Bcl-X_L, Bcl-
W, Bfl-1, and Mcl-1. The second group of pro-apoptotic
20   Bcl-2 family proteins varies widely in their amino-acid
sequences, often containing only a single region of
similarity, the BH3 domain. These "BH3-only" proteins
appear to possess no intrinsic or autonomous
cytodestructive activity, and instead operate as trans-
25   dominant inhibitors of the survival proteins. Their
antagonism of proteins such as Bcl-2 and Bcl-X_L depends on
binding via their BH3 domains to a hydrophobic pocket on
target anti-apoptotic proteins (Kelekar & Thompson,
Trends Cell Biol., 8:324-330 (1998)).

30   Gene knock-out studies in mice have
demonstrated non-redundant roles for various Bcl-2 family
genes in regulating cell life and death in specific
tissues or under particular physiological or pathological

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circumstances (Veis et al., Cell, 75:229-240 (1993);
Motoyama et al., Science, 267:1506-1510 (1995); Knudson
et al., Science, 270:96-99 (1995); Bouillet et al.,
Science, 286:1735-8 (1999); Yin et al., Nature, 400:886-
5   891 (1999)). Thus, it is important to identify all
members of the Bcl-2 family and to delineate the cellular
contexts in which they contribute to apoptosis
regulation. As disclosed herein, a new member of the
Bcl-2 family, Bcl-G, has been cloned and characterized.

10   The nucleic acid molecules described herein are
useful for producing invention proteins, when such
nucleic acids are incorporated into a variety of protein
expression systems known to those of skill in the art.
In addition, such nucleic acid molecules or fragments
15   thereof can be labeled with a readily detectable
substituent and used as hybridization probes for assaying
for the presence and/or amount of an invention Bcl-G gene
or mRNA transcript in a given sample. The nucleic acid
molecules described herein, and fragments thereof, are
20   also useful as primers and/or templates in a PCR reaction
for amplifying genes encoding invention proteins
described herein.

            The term "nucleic acid", also referred to as
polynucleotides, encompasses ribonucleic acid (RNA) or
25   deoxyribonucleic acid (DNA), probes, oligonucleotides,
and primers and can be single stranded or double
stranded. DNA can be either complementary DNA (cDNA) or
genomic DNA, and can represent the sense strand, the
anti-sense strand or both. Examples of nucleic acids are
30   RNA, cDNA, or isolated genomic DNA encoding an Bcl-G
polypeptide. Such nucleic acids include, but are not
limited to, nucleic acids comprising substantially the
same nucleotide sequence as set forth in SEQ ID NOS:1, 3

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or 41. In general, a genomic sequence of the invention includes regulatory regions such as promoters, enhancers, and introns that are outside of the exons encoding a Bcl-G but does not include proximal genes that do not encode

5   Bcl-G.

Use of the terms "isolated" and/or "purified" in the present specification and claims as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have been

10   produced in such form by the hand of man, and thus are separated from their native *in vivo* cellular environment.

As employed herein, the term "substantially the same nucleotide sequence" refers to DNA having sufficient identity to the reference polynucleotide, such that it

15   will hybridize to the reference nucleotide under moderately stringent hybridization conditions. In one embodiment, DNA having substantially the same nucleotide sequence as the reference nucleotide sequence encodes substantially the same amino acid sequence as that set

20   forth in any of SEQ ID NOS:2, 4 or 42. In another embodiment, DNA having "substantially the same nucleotide sequence" as the reference nucleotide sequence has at least 60% identity with respect to the reference nucleotide sequence. DNA having substantially the same

25   nucleotide sequence can have at least 70%, at least 90%, or at least 95% identity to the reference nucleotide sequence.

As used herein, a "modification" of a nucleic acid can also include one or several nucleotide

30   additions, deletions, or substitutions with respect to a reference sequence. A modification of a nucleic acid can include substitutions that do not change the encoded

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amino acid sequence due to the degeneracy of the genetic code. Such modifications can correspond to variations that are made deliberately, or which occur as mutations during nucleic acid replication.

5   Exemplary modifications of the recited Bcl-G sequences include sequences that correspond to homologs of other species, including mammalian species such as mouse, primates, including monkey and baboon, rat, rabbit, bovine, porcine, ovine, canine, feline, or other 10   animal species. The corresponding Bcl-G sequences of non-human species can be determined by methods known in the art, such as by PCR or by screening genomic, cDNA or expression libraries.

          Another exemplary modification of the invention 15   Bcl-G can correspond to splice variant forms of the Bcl-G nucleotide sequence. Additionally, a modification of a nucleotide sequence can include one or more non-native nucleotides, having, for example, modifications to the base, the sugar, or the phosphate portion, or having a 20   modified phosphodiester linkage. Such modifications can be advantageous in increasing the stability of the nucleic acid molecule.

          Furthermore, a modification of a nucleotide sequence can include, for example, a detectable moiety, 25   such as a radiolabel, a fluorochrome, a ferromagnetic substance, a luminescent tag or a detectable binding agent such as biotin. Such modifications can be advantageous in applications where detection of a Bcl-G nucleic acid molecule is desired.

30   The invention also encompasses nucleic acids which differ from the nucleic acids shown in SEQ ID NOS:

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1, 3 or 41, but which have the same phenotype.
Phenotypically similar nucleic acids are also referred to
as "functionally equivalent nucleic acids". As used
herein, the phrase "functionally equivalent nucleic
acids" encompasses nucleic acids characterized by slight
and non-consequential sequence variations that will
function in substantially the same manner to produce the
same protein product(s) as the nucleic acids disclosed
herein. In particular, functionally equivalent nucleic
acids encode polypeptides that are the same as those
encoded by the nucleic acids disclosed herein or that
have conservative amino acid variations. For example,
conservative variations include substitution of a non-
polar residue with another non-polar residue, or
substitution of a charged residue with a similarly
charged residue. These variations include those
recognized by skilled artisans as those that do not
substantially alter the tertiary structure of the
protein.

        Further provided are nucleic acids encoding
Bcl-G polypeptides that, by virtue of the degeneracy of
the genetic code, do not necessarily hybridize to the
invention nucleic acids under specified hybridization
conditions. As used herein, the term "degenerate" refers
to codons that differ in at least one nucleotide from a
reference nucleic acid, but encode the same amino acids
as the reference nucleic acid. Nucleic acids encoding
the invention Bcl-G polypeptides can be comprised of
nucleotides that encode substantially the same amino acid
sequence as set forth in SEQ ID NOS:2, 4 or 42.

        The invention provides an isolated nucleic acid
encoding a Bcl-G polypeptide, or a functional fragment
thereof. The invention also provides an isolated nucleic

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acid encoding a Bcl-G polypeptide, or a functional
fragment thereof, comprising a nucleic acid selected
from:

(a)   nucleic acid encoding the amino acid
sequence set forth in SEQ ID NOS:2, 4 or 42, or

(b)   nucleic acid that hybridizes to the
nucleic acid of (a) under moderately stringent
conditions, wherein said nucleic acid
contiguously encodes biologically active Bcl-G,
or

(c)   nucleic acid degenerate with respect to
either (a) or (b) above, wherein said nucleic
acid encodes biologically active Bcl-G.

In one embodiment, preferred Bcl-G polypeptide
include a long form termed Bcl-G$_L$ and a short form termed
Bcl-G$_S$.   Bcl-G$_L$ contains a BH3 and a BH2 domain, whereas
Bcl-G$_S$ contains only a BH3 domain.   Bcl-G$_S$ has been found
to possess pro-apoptotic activity similar to Bax (see
Example III).

Hybridization refers to the binding of
complementary strands of nucleic acid, for example,
sense:antisense strands or probe:target-nucleic acid to
each other through hydrogen bonds, similar to the bonds
that naturally occur in chromosomal DNA.   Stringency
levels used to hybridize a given probe with target-DNA
can be readily varied by those of skill in the art.

The phrase "stringent hybridization" is used
herein to refer to conditions under which polynucleic
acid hybrids are stable.   As known to those of skill in
the art, the stability of hybrids is reflected in the

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melting temperature (T_m) of the hybrids. In general, the
stability of a hybrid is a function of sodium ion
concentration and temperature. Typically, the
hybridization reaction is performed under conditions of
lower stringency, followed by washes of varying, but
higher, stringency. Reference to hybridization
stringency relates to such washing conditions.

As used herein, the phrase "moderately
stringent hybridization" refers to conditions that permit
target-nucleic acid to bind a complementary nucleic acid.
The hybridized nucleic acids will generally have at least
about 60% identity, at least about 75% identity, more at
least about 85% identity; or at least about 90% identity.
Moderately stringent conditions are conditions equivalent
to hybridization in 50% formamide, 5X Denhart's solution,
5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X
SSPE, 0.2% SDS, at 42°C.

The phrase "high stringency hybridization"
refers to conditions that permit hybridization of only
those nucleic acid sequences that form stable hybrids in
0.018M NaCl at 65°C, for example, if a hybrid is not
stable in 0.018M NaCl at 65°C, it will not be stable
under high stringency conditions, as contemplated herein.
High stringency conditions can be provided, for example,
by hybridization in 50% formamide, 5X Denhart's solution,
5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.1X
SSPE, and 0.1% SDS at 65°C.

The phrase "low stringency hybridization"
refers to conditions equivalent to hybridization in 10%
formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at
22°C, followed by washing in 1X SSPE, 0.2% SDS, at 37°C.
Denhart's solution contains 1% Ficoll, 1%

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polyvinylpyrolidone, and 1% bovine serum albumin (BSA).
20X SSPE (sodium chloride, sodium phosphate, ethylene
diamide tetraacetic acid (EDTA)) contains 3M sodium
chloride, 0.2M sodium phosphate, and 0.025 M (EDTA).
5   Other suitable moderate stringency and high stringency
hybridization buffers and conditions are well known to
those of skill in the art and are described, for example,
in Sambrook et al., _Molecular Cloning: A Laboratory_
Manual, 2nd ed., Cold Spring Harbor Press, Plainview, New
10   York (1989); and Ausubel et al., _supra_, 1999). Nucleic
acids encoding polypeptides hybridize under moderately
stringent or high stringency conditions to substantially
the entire sequence, or substantial portions, for
example, typically at least 15-30 nucleotides of the
15   nucleic acid sequence set forth in SEQ ID NOS:1, 3 or 41.

The invention also provides a modification of a
Bcl-G nucleotide sequence that hybridizes to a Bcl-G
nucleic acid molecule, for example, a nucleic acid
molecule referenced as SEQ ID NOS:1, 3 or 41, under
20   moderately stringent conditions. Modifications of Bcl-G
nucleotide sequences, where the modification has at least
60% identity to a Bcl-G nucleotide sequence, are also
provided. The invention also provides modification of a
Bcl-G nucleotide sequence having at least 65% identity,
25   at least 70% identity, at least 75% identity, at least
80% identity, at least 85% identity, at least 90%
identity, or at least 95% identity.

Identity of any two nucleic acid sequences can
be determined by those skilled in the art based, for
30   example, on a BLAST 2.0 computer alignment, using default
parameters. BLAST 2.0 searching is available at
http://www.ncbi.nlm.nih.gov/gorf/bl2.html., as described
by Tatiana et al., _FEMS Microbiol Lett_. 174:247-250

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(1999); Altschul et al., <u>Nucleic Acids Res.</u>, 25:3389-3402
(1997).

          One means of isolating a nucleic acid encoding
     a Bcl-G polypeptide is to probe a cDNA library or genomic
5   library with a natural or artificially designed nucleic
     acid probe using methods well known in the art. Nucleic
     acid probes derived from the Bcl-G gene are particularly
     useful for this purpose. DNA and cDNA molecules that
     encode Bcl-G polypeptides can be used to obtain
10   complementary genomic DNA, cDNA or RNA from mammals, for
     example, human, mouse, rat, rabbit, pig, and the like, or
     other animal sources, or to isolate related cDNA or
     genomic clones by the screening of cDNA or genomic
     libraries, by methods well known in the art (see, for
15   example, Sambrook et al., supra, 1989; Ausubel et al.,
     supra, 1999).

          The invention additionally provides a nucleic
     acid that hybridizes under high stringency conditions to
     the Bcl-G coding portion of any of SEQ ID NOS:1, 3 or 41.
20   The invention also provides a nucleic acid having a
     nucleotide sequence the same or substantially the same as
     set that forth in any of SEQ ID NOS:1, 3 or 41.

          The invention also provides a method for
     identifying nucleic acids encoding a mammalian Bcl-G by
25   contacting a sample containing nucleic acids with one or
     more Bcl-G oligonucleotides, wherein the contacting is
     effected under high stringency hybridization conditions,
     and identifying a nucleic acid that hybridizes to the
     oligonucleotide. The invention additionally provides a
30   method of detecting a Bcl-G nucleic acid molecule in a
     sample by contacting the sample with two or more Bcl-G

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oligonucleotides, amplifying a nucleic acid molecule, and detecting the amplification. The amplification can be performed, for example, using PCR. The invention further provides oligonucleotides that function as single stranded nucleic acid primers for amplification of a Bcl-G nucleic acid, wherein the primers comprise a nucleic acid sequence derived from the nucleic acid sequences set forth as SEQ ID NOS:1, 3 or 41.

In accordance with a further embodiment of the present invention, optionally labeled Bcl-G-encoding nucleic acids, or fragments thereof, can be employed to probe a library, for example, a cDNA or genomic library, and the like for additional nucleic acid sequences encoding novel Bcl-G polypeptides. Construction of suitable cDNA libraries is well-known in the art. Screening of such a cDNA library is initially carried out under low-stringency conditions, which comprise a temperature of less than about 42°C, a formamide concentration of less than about 50%, and a moderate to low salt concentration.

Presently preferred probe-based screening conditions comprise a temperature of about 37°C, a formamide concentration of about 20%, and a salt concentration of about 5X sodium chloride, sodium citrate (SSC; 20X SSC contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0). Such conditions will allow the identification of sequences having a substantial degree of similarity with the probe sequence, without requiring perfect identity. The phrase "substantial similarity" refers to sequences which share at least 50% identity. Hybridization conditions are selected which allow the identification of sequences having at least 70% identity with the probe, while discriminating against sequences

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having a lower degree of identity with the probe. As a
result, nucleic acids having substantially the same
nucleotide sequence as SEQ ID NOS:1, 3 or 41 are
obtained.

5   As used herein, a nucleic acid "probe" is
single-stranded nucleic acid, or analogs thereof, that
has a sequence of nucleotides that includes at least 14,
at least 20, at least 50, at least 100, at least 200, at
least 300, at least 400, or at least 500 contiguous bases
10   that are the same as or the complement thereof, any
contiguous bases set forth in any of SEQ ID NOS:1, 3 or
41. In addition, the entire cDNA encoding region of an
invention Bcl-G, or the entire sequence corresponding to
SEQ ID NOS:1, 3 or 41 can be used as a probe. Probes can
15   be labeled by methods well-known in the art, as described
hereinafter, and used, for example, in various diagnostic
kits.

            The invention additionally provides a Bcl-G
oligonucleotide comprising between 15 and 300 contiguous
20   nucleotides of SEQ ID NOS:1, 3 or 41, or the anti-sense
strand thereof. As used herein, the term
"oligonucleotide" refers to a nucleic acid molecule that
includes at least 15 contiguous nucleotides from a
reference nucleotide sequence, can include at least 16,
25   17, 18, 19, 20 or at least 25 contiguous nucleotides, and
often includes at least 30, 40, 50, 60, 70, 80, 90, 100,
125, 150, 175, 200, 225, 250, 275, 300, 325, up to 350
contiguous nucleotides from the reference nucleotide
sequence. The reference nucleotide sequence can be the
30   sense strand or the anti-sense strand.

            The Bcl-G oligonucleotides of the invention
that contain at least 15 contiguous nucleotides of a

reference Bcl-G nucleotide sequence are able to hybridize
to Bcl-G under moderately stringent hybridization
conditions and thus can be advantageously used, for
example, as probes to detect Bcl-G DNA or RNA in a

5   sample, and to detect splice variants thereof; as
sequencing or PCR primers; as antisense reagents to block
transcription of Bcl-G RNA in cells; or in other
applications known to those skilled in the art in which
hybridization to a Bcl-G nucleic acid molecule is

10   desirable.

       It is understood that a Bcl-G nucleic acid
molecule, as used herein, specifically excludes
previously known nucleic acid molecules consisting of
nucleotide sequences having identity with the Bcl-G

15   nucleotide sequence (SEQ ID NOS:1, 3 or 41), such as
Expressed Sequence Tags (ESTs), Sequence Tagged Sites
(STSs) and genomic fragments, deposited in public
databases such as the nr, dbest, dbsts, gss and htgs
databases, which are available for searching at

20   http://www.ncbi.nlm.nih.gov/blast/blast.cgi?Jform=0,
using the program BLASTN 2.0.9 described by Altschul et
al., Nucleic Acids Res. 25:3389-3402 (1997).

       In particular, a Bcl-G nucleic acid molecule
specifically excludes nucleic acid molecules consisting

25   of any of the nucleotide sequences having the Genbank
(gb), EMBL (emb) or DDBJ (dbj) accession numbers
described below. Similarly, a Bcl-G polypeptide fragment
specifically excludes the amino acid fragments encoded by
the nucleotide sequences having the GenBank accession

30   numbers described below. GenBank accession numbers
specifically excluded include AC005903, AC007439,
AW000827, AA399486, AW001213, AI478889, AA400686,
AA398276, AI240211, and AA536718. The human BAC

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referenced as GenBank accession No. AC007537 is also
specifically excluded from a Bcl-G nucleic acid.

5   The isolated Bcl-G nucleic acid molecules of
the invention can be used in a variety of diagnostic and
therapeutic applications. For example, the isolated Bcl-
G nucleic acid molecules of the invention can be used as
probes, as described above; as templates for the
recombinant expression of Bcl-G polypeptides; or in
screening assays such as two-hybrid assays to identify
10   cellular molecules that bind Bcl-G.

          Another useful method for producing a Bcl-G
nucleic acid molecule of the invention involves
amplification of the nucleic acid molecule using PCR and
Bcl-G oligonucleotides and, optionally, purification of
15   the resulting product by gel electrophoresis. Either PCR
or RT-PCR can be used to produce a Bcl-G nucleic acid
molecule having any desired nucleotide boundaries.
Desired modifications to the nucleic acid sequence can
also be introduced by choosing an appropriate
20   oligonucleotide primer with one or more additions,
deletions or substitutions. Such nucleic acid molecules
can be amplified exponentially starting from as little as
a single gene or mRNA copy, from any cell, tissue or
species of interest.

25   The invention thus provides methods for
detecting Bcl-G nucleic acid in a sample. The methods of
detecting Bcl-G nucleic acid in a sample can be either
qualitative or quantitative, as desired. For example,
the presence, abundance, integrity or structure of a Bcl-
30   G can be determined, as desired, depending on the assay
format and the probe used for hybridization or primer
pair chosen for application.

25

Useful assays for detecting Bcl-G nucleic acid
based on specific hybridization with an isolated Bcl-G
nucleic acid molecule are well known in the art and
include, for example, *in situ* hybridization, which can be
5   used to detect altered chromosomal location of the
nucleic acid molecule, altered gene copy number, and RNA
abundance, depending on the assay format used. Other
hybridization assays include, for example, Northern blots
and RNase protection assays, which can be used to
10   determine the abundance and integrity of different RNA
splice variants, and Southern blots, which can be used to
determine the copy number and integrity of DNA. A Bcl-G
hybridization probe can be labeled with any suitable
detectable moiety, such as a radioisotope, fluorochrome,
15   chemiluminescent marker, biotin, or other detectable
moiety known in the art that is detectable by analytical
methods.

Useful assays for detecting a Bcl-G nucleic
acid in a sample based on amplifying a Bcl-G nucleic acid
20   with two or more Bcl-G oligonucleotides are also well
known in the art, and include, for example, qualitative
or quantitative polymerase chain reaction (PCR); reverse-
transcription PCR (RT-PCR); single strand conformational
polymorphism (SSCP) analysis, which can readily identify
25   a single point mutation in DNA based on differences in
the secondary structure of single-strand DNA that produce
an altered electrophoretic mobility upon non-denaturing
gel electrophoresis; and coupled PCR, transcription and
translation assays, such as a protein truncation test, in
30   which a mutation in DNA is determined by an altered
protein product on an electrophoresis gel. Additionally,
the amplified Bcl-G nucleic acid can be sequenced to
detect mutations and mutational hot-spots, and specific
assays for large-scale screening of samples to identify

such mutations can be developed.

The invention further provides an isolated Bcl-
G polypeptide, or a functional fragment thereof, encoded
by a Bcl-G nucleic acid of the invention. For example,
the invention provides a polypeptide comprising the same
or substantially the same amino acid sequence as Bcl-G$_L$
(SEQ ID NO:2) or Bcl-G$_S$ (SEQ ID NO:4). Also provided is a
Bcl-G polypeptide encoded by a nucleotide sequence
comprising the same or substantially the same nucleotide
sequence as set forth in SEQ ID NOs:1 or 3. Additionally
provided is mouse Bcl-G nucleotide sequence set forth as
SEQ ID NO:41 (see Example IX).

Described herein is a new member of the *BCL-2*
gene family in humans, *BCL*-G (see Examples I-VIII). The
BCL-G gene potentially encodes two protein products, Bcl-
G$_L$ and Bcl-G$_S$. Bcl-2 family proteins contain up to four
conserved BH domains. The shorter Bcl-G$_S$ protein contains
only the BH3 domains, similar to several other pro-
apoptotic Bcl-2 family proteins, including Bad, Hrk, Bik,
Bim, Apr, and Egl1 (reviewed in Kelekar & Thompson,
Trends Cell Biol., 8:324-330 (1998); Reed, J. Oncogene,
17:3225-3236 (1998)). In contrast, the longer Bcl-G$_L$
protein contains a BH2 and BH3 domain. No other examples
of Bcl-2 family proteins are known which combine BH2 and
BH3 domain in the absence of BH1. Though the Bad protein
was originally suggested to contain a BH2 domain (Yang et
al., Blood, 84(Suppl.1):373a-380a (1994)), and has been
shown to possess the BH3 domain, inspection of the BH2
region reveals very little similarity of amino-acid
sequence with (Ottilie et al., J. Biol. Chem., 272:30866-
30872 (1997)) other BH2 domains. In contrast, the BH2 of
Bcl-G$_L$ contains a stretch of 8 of 8 residues showing
identity or conservative amino-acid substitutions with

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the BH2 domains of other family members. By comparison,
the Bad sequence reveals only 3 of 8 identical or similar
amino-acids in the same region. Thus, Bcl-G$_L$ defines a
novel structural variant within the Bcl-2 family of

5   apoptosis-regulating proteins.

       The production of different protein isoforms by
alternative mRNA splicing is a common feature of *BCL-2*
family genes, including *BCL-2, Bcl-X, MCL-1, BAX,* and *BIM*
(Tsujimoto & Croce, Proc. Natl .Acad. Sci. USA, 83:5214-

10   5218 (1986); O'Connor et al., EMBO J., 17:384-395 (1998);
Boise et al., Cell, 74:597-608 (1993); Oltvai et al.,
Cell, 74:609-619 (1993); Bingle et al., J. Biol. Chem.,
275:22136-22146 (2000)). Unlike *BCL-X*, which encodes a
longer and short protein, Bcl-X$_L$ and Bcl-X$_S$, possessing

15   anti-apoptotic and pro-apoptotic functions, respectively,
the longer isoform of Bcl-G did not display anti-
apoptotic activity. When over-expressed, Bcl-G$_L$ induced
modest and variable increases in apoptosis, whereas the
shorter Bcl-G$_S$ protein consistently exhibited potent

20   cytotoxic activity. This behavior is reminiscent of the
proteins encoded by the *BIM* gene, which include Bim-short
(Bim$_S$), Bim-long (Bim$_L$) and Bim-Extra-Long (Bim$_{EL}$)
(O'Connor et al., EMBO J., 17:384-395 (1998)). The
longer proteins, Bim$_L$ and Bim$_{EL}$, are sequestered in

25   complexes with dynein light-chain (DLC) in association
with microtubules, thus preventing them from interacting
with target proteins such as Bcl-X$_L$ on the surface of
mitochondria and other organelles (Puthalakath et al.,
Mol. Cell, 3:287-96 (1999)). In contrast, because the

30   shortest isoform, Bim$_S$, does not associate with DLC, it is
free to interact with Bcl-X$_L$, Bcl-2, and other survival
proteins and hence displays far more potent apoptotic
activity when over-expressed in cells. By analogy, the
longer Bcl-G$_L$ protein could be sequestered in an inactive

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complex with an unidentified protein.

Besides interactions with sequestering
proteins, the activity of pro-apoptotic Bcl-2 family
proteins can be suppressed by other mechanisms, including
5   post-translational modifications. For example, the Bad
protein is inactivated by phosphorylation. This protein
can be directly or indirectly phosphorylated by several
protein kinases, including PKA, PKB (Akt), Raf1, and
Pak1, thus preventing it from dimerizing with target
10   proteins such as Bcl-2 and Bcl-X_L (reviewed in Reed, J.
Oncogene, 17:3225-3236 (1998); Datta et al., Genes Dev.,
13:2905-2927 (1999)). The intracellular location of Bad
varies, depending on its phosphorylation state, with
phosphorylated Bad residing in the cytosol and
15   unphosphorylated Bad associated with mitochondria and
other intracellular organelles where Bcl-2 and Bcl-X_L are
located. In this regard, the Bcl-G_L protein contains
candidate phosphorylation sites for protein kinase A
(PKA) and protein kinase C (PKC), including some not
20   found in Bcl-G_S. However, in vivo phosphorylation of Bcl-
G_L has not been observed in pilot experiments.

Another post-translational modification shown
previously to activate latent pro-apoptotic Bcl-2 family
proteins is proteolysis. Specifically, the Bid protein
25   contains a N-terminal domain of ~56 amino-acids that
masks its BH3 domain, reducing its ability to dimerize
with other Bcl-2 family proteins. Upon cleavage by
caspases, however, removal of the N-terminal domain
exposes the BH3 domain and is associated with
30   translocation of Bid from the cytosol to mitochondria,
where it induces cytochrome c release and apoptosis (Li
et al., Cell, 94:491-501 (1998); Luo et al., Cell,
94:481-490 (1998)). While Bcl-G_L contains candidate

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caspase recognition sites, no significant cleavage of Bid
has been observed *in vitro* using purified active caspases
or in cells during apoptosis. It is possible, however,
that a specific caspase not yet tested is capable of
cleaving and activating Bcl-G_L.

Though possessing no hydrophobic region that
might anchor it in membranes, the Bcl-G_S protein was
constitutively associated with intracellular organelles.
Interestingly, removal of the BH3 domain did not
interfere with organellar-targeting of Bcl-G_S, but did
abolish dimerization with Bcl-X_L. Thus, the BH3 domain
apparently is not responsible for association of Bcl-G_S
with intracellular organelles. This BH3-independent
targeting of Bcl-G_S differs from some other "BH3-only"
Bcl-2 family proteins such as Bad, where it has been
observed that removal of the BH3 domain abrogates binding
to anti-apoptotic Bcl-2 family proteins as well as
association with mitochondria (Zha et al., J. Biol.
Chem., 272:24101-24104 (1997)).

The *BCL-G* gene resides on chromosome 12p12, a
region deleted in ~50% of prostate cancers, ~30% of
ovarian cancers, and ~30% of childhood acute lymphocytic
leukemias (ALLs) (Kibel et al., J Urol., 1:192-196
(2000); Aissani et al., Leuk Lymphoma, 34:231-239 (1999);
Hatta et al., Br J Cancer, 75:1256-1262 (1997)). Given
that at least one of the protein products of the *BCL-G*
gene exhibits pro-apoptotic function, it is possible that
BCL-G represents a tumor suppressor gene. However, thus
far, somatic mutations in the exons of *BCL-G* have not
been detected nor evidence of deletion of both *BCL-G*
alleles in tumor cell lines or primary tumor specimens
tested so far. Further studies are required therefore to

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determine whether loss of *BCL-G* expression occurs in
tumors by means other than somatic alterations in gene
structure and DNA sequence, such as changes in gene
methylation or aberrant transcriptional or post-
transcriptional regulation.

Investigation of the tissue-distribution of
Bcl-G$_L$ and Bcl-G$_S$ mRNAs by RT-PCR revealed that Bcl-G$_L$
mRNA is found in several normal adult tissues, whereas
Bcl-G$_S$ was detected only in testis. This finding
indicates tissue-specific regulation of Bcl-G$_S$ mRNA
splicing. Tissue-specific splicing of other Bcl-2 family
mRNAs has been observed previously. For example, Bcl-X
mRNA splicing events which generate the pro-apoptotic
Bcl-X$_S$ protein occur in the thymus during T-cell ontogeny
and in the mammary gland during post-lactation
involution, in association with extensive apoptosis
induction (Boise et al., Cell, 74:597-608 (1993);
Heermeier et al., Mech. Dev., 56:197-207 (1996)).
Additional studies are performed to assess differential
mRNA splicing patterns of Bcl-G transcripts during fetal
development and following various scenarios in the adult
where apoptosis occurs as part of .a normal physiological
response or an abnormal pathological reaction to
environmental insults.

As employed herein, the term "substantially the
same amino acid sequence" refers to amino acid sequences
having at least about 70% identity with respect to the
reference amino acid sequence, and retaining comparable
functional and biological activity characteristic of the
protein defined by the reference amino acid sequence.
Preferably, proteins having "substantially the same amino
acid sequence" will have at least about 80%, more
preferably 90% amino acid identity with respect to the

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reference amino acid sequence; with greater than about
95% amino acid sequence identity being especially
preferred. It is recognized, however, that polypeptides,
or encoding nucleic acids, containing less than the
5   described levels of sequence identity arising as splice
variants or that are modified by conservative amino acid
substitutions, or by substitution of degenerate codons
are also encompassed within the scope of the present
invention.

10   Also encompassed by the term Bcl-G are
functional fragments or polypeptide analogs thereof. The
term "functional fragment" refers to a peptide fragment
that is a portion of a full length Bcl-G protein,
provided that the portion has a biological activity, as
15   defined herein, that is characteristic of the
corresponding full length protein. Thus, the invention
also provides functional fragments of invention Bcl-G
proteins, which can be identified using the binding and
routine methods, such as bioassays described herein. A
20   Bcl-G polypeptide functional fragment can be a BH3 or BH2
domain, for example, a BH3 domain referenced as SEQ ID
NOS:5 or 9 or a BH2 domain referenced as SEQ ID NOS:6 or
18. The BH3 domain of Bcl-G is 33% identical to the BH3
domain of Bcl-2, 44% identical to the BH3 domain of Bcl-
25   X_L, and 66% identical to the BH3 domain of Bax.

                In addition, a functional fragment of a Bcl-G
polypeptide can be Bax homology region. A region
upstream of the BH3 domain shares a high degree of
homology with Bax, including a 12 amino acid residue
30   motif that is 70% identical between Bcl-G and Bax.
Therefore, such a Bax homology region can function
similarly to Bax, for example, as a possible binding
domain. The N-terminal 150 amino acids of Bcl-G are not

similar to any known amino acid sequence available in public databases. Therefore, the N-terminal region of Bcl-G can function as Bcl-G-specific functional domain that confers a biological activity that is specific for

5   Bcl-G relative to other members of the Bcl-2 family.

The invention also provides a chimeric protein comprising a domain selected from the group consisting of BH3 (SEQ ID NOS:5 or 9) and BH2 (SEQ ID NOS:6 or 18). A chimeric protein comprising a Bcl-G functional domain can

10   be generated, for example, by recombinantly expressing a Bcl-G domain such as BH2 or BH3 fused to another polypeptide. Alternatively, the Bcl-G functional domain can be expressed as a fusion to another polypeptide.

In another embodiment of the invention, Bcl-G-

15   containing chimeric proteins are provided comprising an invention Bcl-G, or fragments thereof, having the sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:42, and further comprising one or more sequences from a heterologous protein. Sequences from heterologous

20   proteins with which the Bcl-G or functional fragment thereof are fused can include, for example, glutathione-S-transferase, an antibody, or other proteins or functional fragments thereof which facilitate recovery of the chimera. Further proteins with which the Bcl-G or

25   functional fragment thereof are fused will include, for example, luciferase, green fluorescent protein, an antibody, or other proteins or functional fragments thereof which facilitate identification of the chimera. Still further proteins with which the Bcl-G or functional

30   fragment thereof are fused will include, for example, the LexA DNA binding domain, ricin, α-sarcin, an antibody, or other proteins which have therapeutic properties or other biological activity.

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As such chimeric proteins include sequences
from two different proteins, the resultant amino acid
sequence of the chimeric protein will typically be a non-
naturally occurring sequence. Thus, in accordance with
5   this embodiment of the invention, there are provided
chimeric proteins comprising an invention Bcl-G, or
fragments thereof, having the sequence of SEQ ID NO:2,
SEQ ID NO:4, or SEQ ID NO:42, or a fragment thereof,
provided the sequence of the chimeric protein is not
10   naturally occurring.

In another embodiment of the invention, there
are provided hetero-oligomers comprising invention Bcl-G
polypeptides and fragments thereof, invention Bcl-G-
containing proteins, Bcl-G-containing chimeric proteins,
15   or combinations thereof. As disclosed herein, Bcl-G
contains a BH3 domain, which functions as a ligand to
bind Bcl-2 family members (Example I). Bcl-G can
function to bind Bcl-2 family members. Thus, hetero-
oligomers comprising invention Bcl-G polypeptides (SEQ ID
20   NOS:2, 4 or 42) and fragments thereof, invention Bcl-G-
containing proteins, Bcl-G-containing chimeric proteins,
or combinations thereof, and further comprising Bcl-2
family members such as Bcl-2, Bcl-X$_L$ or other Bcl-2 family
members are provided.

25   As used herein, the term "polypeptide" when
used in reference to Bcl-G is intended to refer to a
peptide or polypeptide of two or more amino acids. The
term "polypeptide analog" includes any polypeptide having
an amino acid residue sequence substantially the same as
30   a sequence specifically described herein in which one or
more residues have been conservatively substituted with a
functionally similar residue and which displays the
ability to functionally mimic a Bcl-G as described

herein. A "modification" of a Bcl-G polypeptide also
encompasses conservative substitutions of a Bcl-G
polypeptide amino acid sequence. Conservative
substitutions of encoded amino acids include, for
example, amino acids that belong within the following
groups: (1) non-polar amino acids (Gly, Ala, Val, Leu,
and Ile); (2) polar neutral amino acids (Cys, Met, Ser,
Thr, Asn, and Gln); (3) polar acidic amino acids (Asp and
Glu); (4) polar basic amino acids (Lys, Arg and His); and
(5) aromatic amino acids (Phe, Trp, Tyr, and His). Other
minor modifications are included within Bcl-G
polypeptides so long as the polypeptide retains some or
all of its function as described herein.

The amino acid length of functional fragments
or polypeptide anlogs of the present invention can range
from about 5 amino acids up to the full-length protein
sequence of an invention Bcl-G. In certain embodiments,
the amino acid lengths include, for example, at least
about 10 amino acids, at least about 15, at least about
20, at least about 25, at least about 30, at least about
35, at least about 40, at least about 45, at least about
50, at least about 75, at least about 100, at least about
150, at least about 200, at least about 250 or more amino
acids in length up to the full-length Bcl-G protein
sequence. The functional fragments can be contiguous
amino acid sequences of a Bcl-G polypeptide, including
contiguous amino acid sequences of SEQ ID NOS:2, 4 or 42.

A modification of a polypeptide can also
include derivatives, analogues and functional mimetics
thereof, provided that such polypeptide displays the Bcl-
G biological activity. For example, derivatives can
include chemical modifications of the polypeptide such as
alkylation, acylation, carbamylation, iodination, or any

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modification that derivatizes the polypeptide. Such
derivatized molecules include, for example, those
molecules in which free amino groups have been
derivatized to form amine hydrochlorides, p-toluene
sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl
groups, chloroacetyl groups or formyl groups. Free
carboxyl groups can be derivatized to form salts, methyl
and ethyl esters or other types of esters or hydrazides.
Free hydroxyl groups can be derivatized to form O-acyl or
O-alkyl derivatives. The imidazole nitrogen of histidine
can be derivatized to form N-im-benzylhistidine. Also
included as derivatives or analogues are those peptides
which contain one or more naturally occurring amino acid
derivatives of the twenty standard amino acids, for
example, 4-hydroxyproline, 5-hydroxylysine,
3-methylhistidine, homoserine, ornithine or
carboxyglutamate, and can include amino acids that are
not linked by peptide bonds. Polypeptides of the present
invention also include any polypeptide having one or more
additions and/or deletions of residues, relative to the
sequence of a polypeptide whose sequence is shown herein,
so long as Bcl-G activity is maintained.

    A modification of a Bcl-G polypeptide includes
functional mimetics thereof. Mimetics encompass
chemicals containing chemical moieties that mimic the
function of the polypeptide. For example, if a
polypeptide contains two charged chemical moieties having
functional activity, a mimetic places two charged
chemical moieties in a spatial orientation and
constrained structure so that the charged chemical
function is maintained in three-dimensional space. Thus,
a mimetic, which orients functional groups that provide a
function of Bcl-G, are included within the meaning of a
Bcl-G derivative. All of these modifications are

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included within the term "polypeptide" so long as the Bcl-G polypeptide or functional fragment retains its function.

The invention provides an isolated Bcl-G polypeptide, or functional fragment thereof. The invention Bcl-G polypeptides can be isolated by a variety of methods well-known in the art, for example, recombinant expression systems described herein, precipitation, gel filtration, ion-exchange, reverse-phase and affinity chromatography, and the like. Other well-known methods are described in Deutscher et al., Guide to Protein Purification: Methods in Enzymology Vol. 182, (Academic Press, (1990)). Alternatively, the isolated polypeptides of the present invention can be obtained using well-known recombinant methods (see, for example, Sambrook et al., *supra*, 1989; Ausubel et al., *supra*, 1999). The methods and conditions for biochemical purification of a polypeptide of the invention can be chosen by those skilled in the art, and purification monitored, for example, by an immunological assay or a functional assay.

An example of the means for preparing the invention polypeptide(s) is to express nucleic acids encoding Bcl-G in a suitable host cell, such as a bacterial cell, a yeast cell, an amphibian cell such as an oocyte, or a mammalian cell, using methods well known in the art, and recovering the expressed polypeptide, again using well-known purification methods, so described herein. Invention polypeptides can be isolated directly from cells that have been transformed with expression vectors as described herein. Recombinantly expressed polypeptides of the invention can also be expressed as fusion proteins with appropriate affinity tags, such as

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glutathione S transferase (GST) or poly His, and affinity purified. The invention polypeptide, biologically functional fragments, and functional equivalents thereof can also be produced by chemical synthesis. For example, synthetic polypeptides can be produced using Applied Biosystems, Inc. Model 430A or 431A automatic peptide synthesizer (Foster City, CA) employing the chemistry provided by the manufacturer.

Bcl-G polypeptides can be administered to an individual to increase an activity associated with a Bcl-G polypeptide, including induction of apoptosis or functioning as a tumor suppressor. For example, a Bcl-G polypeptide can be administered therapeutically to an individual using expression vectors containing nucleic acids encoding Bcl-G polypeptides, as described below. In addition, Bcl-G polypeptides, or a functional portion thereof, can be directly administered to an individual. Methods of administering therapeutic polypeptides are well known to those skilled in the art, for example, as a pharmaceutical composition.

In a particular embodiment, a Bcl-G polypeptide, or functional fragment thereof, can be administered to an individual so that the Bcl-G polypeptide or functional fragment is targeted to a tumor to induce apoptosis or otherwise function as a tumor suppressor. One method of delivering a Bcl-G polypeptide to an intracellular target is to fuse a Bcl-G polypeptide or functional fragment to an intracellular-targeting peptide that can penetrate the cell membrane or otherwise deliver a polypeptide to the intracellular environment such as via internalization, thereby causing the fused Bcl-G polypeptide to enter the cell. One example of such an intracellular-targeting peptides is a fusion to the

transduction domain of HIV TAT, which allows transduction of up to 100% of cells (Schwarze et al., Science 285:1569-1572 (1999); Vocero-Akbani et al., Nature Med. 5:29-33 (1999)).

5   Another example of such an intracellular-targeting peptide is the Antennapeida homeoprotein internalization domain (Holinger et al., J. Biol. Chem. 274:13298-13304 (1999)). Still another intracellular-targeting peptide is a peptide that is specific for a
10   cell surface receptor, which allows binding and internalization of a fusion polypeptide via receptor-mediated endocytosis (Ellerby et al., Nature Med. 5:1032-1038 (1999)). Such intracellular-targeting peptides that mediate specific receptor interactions can be
15   advantageously used to target a tumor (see Ellerby et al., supra, 1999). Alternatively, a Bcl-G polypeptide of the invention can be incorporated, if desired, into liposomes, microspheres or other polymer matrices (Gregoriadis, Liposome Technology, Vols. I to III,
20   2nd ed., CRC Press, Boca Raton FL (1993)).

         The invention additionally provides a method for modulating the activity of an oncogenic polypeptide by contacting the oncogenic polypeptide with a substantially pure Bcl-G, or an oncogenic protein-binding
25   fragment thereof. Bcl-G can function to bind oncogenic proteins such as Bcl-2. Therefore, Bcl-G or functional fragments that bind to an oncogenic protein such as Bcl-2 can be used to modulate the activity of the oncogenic protein.

30   The present invention also provides compositions containing an acceptable carrier and any of an isolated, purified Bcl-G mature protein or functional

polypeptide fragments thereof, alone or in combination
with each other. These polypeptides or proteins can be
recombinantly derived, chemically synthesized or purified
from native sources. As used herein, the term
5   "acceptable carrier" encompasses any of the standard
pharmaceutical carriers, such as phosphate buffered
saline solution, water and emulsions such as an oil and
water emulsion, and various types of wetting agents.

The invention thus provides a therapeutic
10   composition comprising a pharmaceutically acceptable
carrier and a compound selected from the group consisting
of a Bcl-G polypeptide, a functional fragment of Bcl-G, a
Bcl-G modulating compound, and an anti-Bcl-G antibody.
The invention additionally provides a method of treating
15   a pathology characterized by abnormal cell proliferation
by administering an effective amount of the composition
containing a pharmaceutically acceptable carrier and a
compound selected from the group consisting of a Bcl-G
polypeptide, a functional fragment of Bcl-G, a Bcl-G
20   modulating compound, and an anti-Bcl-G antibody.

Also provided are antisense-nucleic acids
having a sequence capable of binding specifically with
full-length or any portion of an mRNA that encodes Bcl-G
polypeptides so as to prevent translation of the mRNA.
25   The antisense-nucleic acid can have a sequence capable of
binding specifically with any portion of the sequence of
the cDNA encoding Bcl-G polypeptides. As used herein,
the phrase "binding specifically" encompasses the ability
of a nucleic acid sequence to recognize a complementary
30   nucleic acid sequence and to form double-helical segments
therewith via the formation of hydrogen bonds between the
complementary base pairs. An example of an antisense-
nucleic acid is an antisense-nucleic acid comprising

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chemical analogs of nucleotides.

The present invention provides means to
modulate levels of expression of Bcl-G polypeptides by
recombinantly expressing Bcl-G anti-sense nucleic acids
5   or employing synthetic anti-sense nucleic acid
compositions (hereinafter SANC) that inhibit translation
of mRNA encoding these polypeptides. Synthetic
oligonucleotides, or other antisense-nucleic acid
chemical structures designed to recognize and selectively
10   bind to mRNA are constructed to be complementary to full-
length or portions of an Bcl-G coding strand, including
nucleotide sequences set forth in SEQ ID NOS:1, 3 or 41.

The SANC is designed to be stable in the blood
stream for administration to a subject by injection, or
15   in laboratory cell culture conditions. The SANC is
designed to be capable of passing through the cell
membrane in order to enter the cytoplasm of the cell by
virtue of physical and chemical properties of the SANC,
which render it capable of passing through cell
20   membranes, for example, by designing small, hydrophobic
SANC chemical structures, or by virtue of specific
transport systems in the cell which recognize and
transport the SANC into the cell. In addition, the SANC
can be designed for administration only to certain
25   selected cell populations by targeting the SANC to be
recognized by specific cellular uptake mechanisms which
bind and take up the SANC only within select cell
populations. In a particular embodiment the SANC is an
antisense oligonucleotide.

30   For example, the SANC may be designed to bind
to a receptor found only in a certain cell type, as
discussed above. The SANC is also designed to recognize

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and selectively bind to target mRNA sequence, which can
correspond to a sequence contained within the sequences
shown in SEQ ID NOS:1, 3 or 41. The SANC is designed to
inactivate target mRNA sequence by either binding thereto
5   and inducing degradation of the mRNA by, for example,
RNase I digestion, or inhibiting translation of mRNA
target sequence by interfering with the binding of
translation-regulating factors or ribosomes, or inclusion
of other chemical structures, such as ribozyme sequences
10   or reactive chemical groups which either degrade or
chemically modify the target mRNA. SANCs have been shown
to be capable of such properties when directed against
mRNA targets (see Cohen et al., TIPS, 10:435 (1989) and
Weintraub, Sci. American, January (1990), pp.40).

15   The invention further provides a method of
modulating the level of apoptosis in a cell by
introducing an antisense nucleotide sequence into the
cell, wherein the antisense nucleotide sequence
specifically hybridizes to a nucleic acid molecule
20   encoding a Bcl-G, wherein the hybridization reduces or
inhibits the expression of the Bcl-G in the cell. The
use of anti-sense nucleic acids, including recombinant
anti-sense nucleic acids or SANCs, can be advantageously
used to inhibit cell death.

25   Compositions comprising an amount of the
antisense-nucleic acid of the invention, effective to
reduce expression of Bcl-G polypeptides by entering a
cell and binding specifically to mRNA encoding Bcl-G
polypeptides so as to prevent translation and an
30   acceptable hydrophobic carrier capable of passing through
a cell membrane are also provided herein. Suitable
hydrophobic carriers are described, for example, in U.S.
Patent Nos. 5,334,761; 4,889,953; 4,897,355, and the

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like. The acceptable hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of

5   the selected cell type. For example, the structure can be part of a protein known to bind to a cell-type specific receptor such as a tumor.

       Antisense-nucleic acid compositions are useful to inhibit translation of mRNA encoding invention

10   polypeptides. Synthetic oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding Bcl-G polypeptides and inhibit translation of mRNA and are useful as compositions to inhibit expression of Bcl-G associated genes in a tissue sample

15   or in a subject.

       The invention also provides a method for expression of a Bcl-G polypeptide by culturing cells containing a Bcl-G nucleic acid under conditions suitable for expression of Bcl-G. Thus, there is provided a

20   method for the recombinant production of a Bcl-G of the invention by expressing the nucleic acid sequences encoding Bcl-G in suitable host cells. Recombinant DNA expression systems that are suitable to produce Bcl-G described herein are well-known in the art (see, for

25   example, Ausubel et al., *supra*, 1999). For example, the above-described nucleotide sequences can be incorporated into vectors for further manipulation. As used herein, vector refers to a recombinant DNA or RNA plasmid or virus containing discrete elements that are used to

30   introduce heterologous DNA into cells for either expression or replication thereof.

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        The invention also provides vectors containing
the Bcl-G nucleic acids of the invention. Suitable
expression vectors are well-known in the art and include
vectors capable of expressing nucleic acid operatively
5   linked to a regulatory sequence or element such as a
promoter region or enhancer region that is capable of
regulating expression of such nucleic acid. Appropriate
expression vectors include those that are replicable in
eukaryotic cells and/or prokaryotic cells and those that
10   remain episomal or those which integrate into the host
cell genome.

        Promoters or enhancers, depending upon the
nature of the regulation, can be constitutive or
regulated. The regulatory sequences or regulatory
15   elements are operatively linked to a nucleic acid of the
invention such that the physical and functional
relationship between the nucleic acid and the regulatory
sequence allows transcription of the nucleic acid.

        Suitable vectors for expression in prokaryotic
20   or eukaryotic cells are well known to those skilled in
the art (see, for example, Ausubel et al., supra, 1999).
Vectors useful for expression in eukaryotic cells can
include, for example, regulatory elements including the
SV40 early promoter, the cytomegalovirus (CMV) promoter,
25   the mouse mammary tumor virus (MMTV) steroid-inducible
promoter, Moloney murine leukemia virus (MMLV) promoter,
and the like. The vectors of the invention are useful
for subcloning and amplifying a Bcl-G nucleic acid
molecule and for recombinantly expressing a Bcl-G
30   polypeptide. A vector of the invention can include, for
example, viral vectors such as a bacteriophage, a
baculovirus or a retrovirus; cosmids or plasmids; and,
particularly for cloning large nucleic acid molecules,

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bacterial artificial chromosome vectors (BACs) and yeast
artificial chromosome vectors (YACs). Such vectors are
commercially available, and their uses are well known in
the art. One skilled in the art will know or can readily
5   determine an appropriate promoter for expression in a
particular host cell.

The invention additionally provides recombinant
cells containing Bcl-G nucleic acids of the invention.
The recombinant cells are generated by introducing into a
10   host cell a vector containing a Bcl-G nucleic acid
molecule. The recombinant cells are transducted,
transfected or otherwise genetically modified. Exemplary
host cells that can be used to express recombinant Bcl-G
molecules include mammalian primary cells; established
15   mammalian cell lines, such as COS, CHO, HeLa, NIH3T3, HEK
293 and PC12 cells; amphibian cells, such as *Xenopus*
embryos and oocytes; and other vertebrate cells.
Exemplary host cells also include insect cells such as
Drosophila, yeast cells such as *Saccharomyces cerevisiae*,
20   *Saccharomyces pombe*, or *Pichia pastoris*, and prokaryotic
cells such as *Escherichia coli*.

In one embodiment, nucleic acids encoding the
invention Bcl-G polypeptides can be delivered into
mammalian cells, either *in vivo* or *in vitro* using
25   suitable vectors well-known in the art. Suitable vectors
for delivering a Bcl-G polypeptide, or a functional
fragment thereof to a mammalian cell, include viral
vectors such as retroviral vectors, adenovirus, adeno-
associated virus, lentivirus, herpesvirus, as well as
30   non-viral vectors such as plasmid vectors. Such vectors
are useful for providing therapeutic amounts of a Bcl-G
polypeptide (see, for example, U.S. Patent No. 5,399,346,
issued March 21, 1995). Delivery of Bcl-G polypeptides

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or nucleic acids therapeutically can be particularly
useful when targeted to a tumor cell, thereby inducing
apoptosis in tumor cells. In addition, where it is
desirable to limit or reduce the *in vivo* expression of
5   the invention Bcl-G, the introduction of the antisense
strand of the invention nucleic acid is contemplated.

Viral based systems provide the advantage of
being able to introduce relatively high levels of the
10   heterologous nucleic acid into a variety of cells.
Suitable viral vectors for introducing invention nucleic
acid encoding an Bcl-G protein into mammalian cells are
well known in the art. These viral vectors include, for
example, Herpes simplex virus vectors (Geller et al.,
15   Science, 241:1667-1669 (1988)); vaccinia virus vectors
(Piccini et al., Meth. Enzymology, 153:545-563 (1987));
cytomegalovirus vectors (Mocarski et al., in Viral
Vectors, Y. Gluzman and S.H. Hughes, Eds., Cold Spring
Harbor Laboratory, Cold Spring Harbor, N.Y., 1988, pp.
20   78-84)); Moloney murine leukemia virus vectors (Danos et
al., Proc. Natl. Acad. Sci. USA, 85:6460-6464 (1988);
Blaese et al., Science, 270:475-479 (1995); Onodera et
al., J. Virol., 72:1769-1774 (1998)); adenovirus vectors
(Berkner, Biotechniques, 6:616-626 (1988); Cotten et al.,
25   Proc. Natl. Acad. Sci. USA, 89:6094-6098 (1992); Graham
et al., Meth. Mol. Biol., 7:109-127 (1991); Li et al.,
Human Gene Therapy, 4:403-409 (1993); Zabner et al.,
Nature Genetics, 6:75-83 (1994)); adeno-associated virus
vectors (Goldman et al., Human Gene Therapy, 10:2261-2268
30   (1997); Greelish et al., Nature Med., 5:439-443 (1999);
Wang et al., Proc. Natl. Acad. Sci. USA, 96:3906-3910
(1999); Snyder et al., Nature Med., 5:64-70 (1999);
Herzog et al., Nature Med., 5:56-63 (1999)); retrovirus
vectors (Donahue et al., Nature Med., 4:181-186 (1998);
35   Shackleford et al., Proc. Natl. Acad. Sci. USA,

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85:9655-9659 (1988); U.S. Patent Nos. 4,405,712,
4,650,764 and 5,252,479, and WIPO publications WO
92/07573, WO 90/06997, WO 89/05345, WO 92/05266 and WO
92/14829; and lentivirus vectors (Kafri et al., Nature

5   Genetics, 17:314-317 (1997)).

For example, in one embodiment of the present
invention, adenovirus-transferrin/polylysine-DNA
(TfAdpl-DNA) vector complexes (Wagner et al., Proc. Natl.
Acad. Sci., USA, 89:6099-6103 (1992); Curiel et al., Hum.

10   Gene Ther., 3:147-154 (1992); Gao et al., Hum. Gene
Ther., 4:14-24 (1993)) are employed to transduce
mammalian cells with heterologous Bcl-G nucleic acid.
Any of the plasmid expression vectors described herein
may be employed in a TfAdpl-DNA complex.

15   Vectors useful for therapeutic administration
of a Bcl-G polypeptide of nucleic acid can contain a
regulatory element that provides tissue specific or
inducible expression of an operatively linked nucleic
acid. One skilled in the art can readily determine an

20   appropriate tissue-specific promotor or enhancer that
allows exparssion of a Bcl-G polypeptide or nucleic acid
in a desired tissue. Any of a variety of inducible
promoters or enhancers can also be included in the vector
for regulatable expression of a Bcl-G polypeptide or

25   nucleic acid. Such inducible systems, include, for
example, tetracycline inducible system (Gossen & Bizard,
Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992); Gossen
et al., Science, 268:1766-1769 (1995); Clontech, Palo
Alto, CA); metalothionein promoter induced by heavy

30   metals; insect steroid hormone responsive to ecdysone or
related steroids such as muristerone (No et al., Proc.
Natl. Acad. Sci. USA, 93:3346-3351 (1996); Yao et al.,
Nature, 366:476-479 (1993); Invitrogen, Carlsbad, CA);

mouse mammory tumor virus (MMTV) induced by steroids such
as glucocortocoid and estrogen (Lee et al., Nature,
294:228-232 (1981); and heat shock promoters inducible by
temperature changes.

An inducible system particularly useful for
therapeutic administration utilizes an inducible promotor
that can be regulated to deliver a level of therapeutic
product in response to a given level of drug administered
to an individual and to have little or no expression of
the therapeutic product in the absence of the drug. One
such system utilizes a Gal4 fusion that is inducible by
an antiprogestin such as mifepristone in a modified
adenovirus vector (Burien et al., Proc. Natl. Acad. Sci.
USA, 96:355-360 (1999). Another such inducible system
utilizes the drug rapamycin to induce reconstitution of a
transcriptional activator containing rapamycin binding
domains of FKBP12 and FRAP in an adeno-associated virus
vector (Ye et al., Science, 283:88-91 (1999)). It is
understood that any combination of an inducible system
can be combined in any suitable vector, including those
disclosed herein. Such a regulatable inducible system is
advantageous because the level of expression of the
therapeutic product can be controlled by the amount of
drug administered to the individual or, if desired,
expression of the therapeutic product can be terminated
by stopping administration of the drug.

The invention additionally provides an isolated
anti-Bcl-G antibody having specific reactivity with a
Bcl-G. The anti-Bcl-G antibody can be a monoclonal
antibody or a polyclonal antibody. The invention further
provides cell lines producing monoclongal antibodies
having specific reactivity with a Bcl-G.

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The invention thus provides antibodies that specifically bind a Bcl-G polypeptide. As used herein, the term "antibody" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as antigen binding fragments of such antibodies. With regard to an anti-Bcl-G antibody of the invention, the term "antigen" means a native or synthesized Bcl-G polypeptide or fragment thereof. An anti-Bcl-G antibody, or antigen binding fragment of such an antibody, is characterized by having specific binding activity for a Bcl-G polypeptide or a peptide portion thereof of at least about 1×10^5 M^{-1}. Thus, Fab, F(ab')$_2$, Fd and Fv fragments of an anti-Bcl-G antibody, which retain specific binding activity for a Bcl-G polypeptide, are included within the definition of an antibody. Specific binding activity of a Bcl-G polypeptide can be readily determined by one skilled in the art, for example, by comparing the binding activity of an anti-Bcl-G antibody to a Bcl-G polypeptide versus a control polypeptide that is not a Bcl-G polypeptide. Methods of preparing polyclonal or monoclonal antibodies are well known to those skilled in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1988)).

In addition, the term "antibody" as used herein includes naturally occurring antibodies as well as non-naturally occurring antibodies, including, for example, single chain antibodies, chimeric, bifunctional and humanized antibodies, as well as antigen-binding fragments thereof. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and

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variable light chains as described by Huse et al. (<u>Science</u> 246:1275-1281 (1989)). These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bifunctional antibodies are well known to those skilled in the art (Winter and Harris, <u>Immunol. Today</u> 14:243-246 (1993); Ward et al., <u>Nature</u> 341:544-546 (1989) ; Harlow and Lane, *supra*, 1988); Hilyard et al., <u>Protein Engineering: A practical approach</u> (IRL Press 1992); Borrabeck, <u>Antibody Engineering</u>, 2d ed. (Oxford University Press 1995)).

Anti-Bcl-G antibodies can be raised using a Bcl-G immunogen such as an isolated Bcl-G polypeptide having the amino acid sequence of SEQ ID NOS:2, 4 or 42, or a fragment thereof, which can be prepared from natural sources or produced recombinantly, or a peptide portion of the Bcl-G polypeptide. Such peptide portions of a Bcl-G polypeptide are functional antigenic fragments if the antigenic peptides can be used to generate a Bcl-G-specific antibody. A non-immunogenic or weakly immunogenic Bcl-G polypeptide or portion thereof can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art (see, for example, Harlow and Lane, *supra*, 1988). An immunogenic Bcl-G polypeptide fragment can also be generated by expressing the peptide portion as a fusion protein, for example, to glutathione S transferase (GST), polyHis or the like. Methods for expressing peptide fusions are well known to those skilled in the art (Ausubel et al., <u>Current Protocols in Molecular Biology</u> (Supplement 47), John Wiley & Sons, New York (1999)).

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The invention further provides a method for detecting the presence of a human Bcl-G in a sample by contacting a sample with a Bcl-G-specific antibody, and detecting the presence of specific binding of the antibody to the sample, thereby detecting the presence of a human Bcl-G in the sample. Bcl-G specific antibodies can be used in diagnostic methods and systems to detect the level of Bcl-G present in a sample. As used herein, the term "sample" is intended to mean any biological fluid, cell, tissue, organ or portion thereof, that includes or potentially includes Bcl-G nucleic acids or polypeptides. The term includes samples present in an individual as well as samples obtained or derived from the individual. For example, a sample can be a histologic section of a specimen obtained by biopsy, or cells that are placed in or adapted to tissue culture. A sample further can be a subcellular fraction or extract, or a crude or substantially pure nucleic acid or protein preparation.

Bcl-G-specific antibodies can also be used for the immunoaffinity or affinity chromatography purification of the invention Bcl-G. In addition, methods are contemplated herein for detecting the presence of an invention Bcl-G protein in a cell, comprising contacting the cell with an antibody that specifically binds to Bcl-G polypeptides under conditions permitting binding of the antibody to the Bcl-G polypeptides, detecting the presence of the antibody bound to the Bcl-G polypeptide, and thereby detecting the presence of invention polypeptides in a cell. With respect to the detection of such polypeptides, the antibodies can be used for *in vitro* diagnostic or *in vivo* imaging methods.

Immunological procedures useful for *in vitro*
detection of target Bcl-G polypeptides in a sample
include immunoassays that employ a detectable antibody.
Such immunoassays include, for example,
5   immunohistochemistry, immunofluorescence, ELISA assays,
radioimmunoassay, FACS analysis, immunoprecipitation,
immunoblot analysis, Pandex microfluorimetric assay,
agglutination assays, flow cytometry and serum diagnostic
assays, which are well known in the art (Harlow and Lane,
10   *supra*, 1988; Harlow and Lane, Using Antibodies: A
Laboratory Manual, Cold Spring Harbor Press (1999)).

An antibody can be made detectable by various
means well known in the art. For example, a detectable
marker can be directly attached to the antibody or
15   indirectly attached using, for example, a secondary agent
that recognizes the Bcl-G specific antibody. Useful
markers include, for example, radionucleotides, enzymes,
binding proteins such as biotin, fluorogens, chromogens
and chemiluminescent labels.

20   As used herein, the terms "label" and
"indicating means" in their various grammatical forms
refer to single atoms and molecules that are either
directly or indirectly involved in the production of a
detectable signal. Any label or indicating means can be
25   linked to invention nucleic acid probes, expressed
proteins, polypeptide fragments, or antibody molecules.
These atoms or molecules can be used alone or in
conjunction with additional reagents. Such labels are
themselves well-known in clinical diagnostic chemistry.

30   The labeling means can be a fluorescent
labeling agent that chemically binds to antibodies or
antigens without denaturation to form a fluorochrome

(dye) that is a useful immunofluorescent tracer. A description of immunofluorescent analytic techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, Marchalonis et al., eds., John Wiley

5   & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

        In one embodiment, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, and the like. In another embodiment,

10 radioactive elements are employed labeling agents. The linking of a label to a substrate, i.e., labeling of nucleic acid probes, antibodies, polypeptides, and proteins, is well known in the art. For instance, an invention antibody can be labeled by metabolic

15 incorporation of radiolabeled amino acids provided in the culture medium. See, for example, Galfre et al., Meth. Enzymol., 73:3-46 (1981). Conventional means of protein conjugation or coupling by activated functional groups are particularly applicable. See, for example, Aurameas

20 et al., Scand. J. Immunol., Vol. 8, Suppl. 7:7-23 (1978), Rodwell et al., Biotech., 3:889-894 (1984), and U.S. Patent No. 4,493,795.

        In addition to detecting the presence of a Bcl-G polypeptide, invention anti-Bcl-G antibodies are

25 contemplated for use herein to modulate the activity of the Bcl-G polypeptide in living animals, in humans, or in biological tissues or fluids isolated therefrom. The term "modulate" refers to a compound's ability to increase the biological activity by functioning as an

30 agonist or inhibit the biological activity by functioning as an antagonist of an invention Bcl-G polypeptide. Accordingly, compositions comprising a carrier and an amount of an antibody having specificity for Bcl-G

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polypeptides effective to block naturally occurring
ligands or other Bcl-G-binding proteins from binding to
invention Bcl-G polypeptides are contemplated herein.
For example, a monoclonal antibody directed to an epitope
of an invention Bcl-G polypeptide, including an amino
acid sequence set forth in SEQ ID NOS:2, 4 or 42, can be
useful for this purpose.

The present invention further provides
transgenic non-human mammals that are capable of
expressing exogenous nucleic acids encoding Bcl-G
polypeptides. As employed herein, the phrase "exogenous
nucleic acid" refers to nucleic acid sequence which is
not native to the host, or which is present in the host
in other than its native environment, for example, as
part of a genetically engineered DNA construct. In
addition to naturally occurring levels of Bcl-G, a Bcl-G
polypeptide of the invention can either be overexpressed
or underexpressed in transgenic mammals, for example,
underexpressed in a knock-out animal.

Also provided are transgenic non-human mammals
capable of expressing nucleic acids encoding Bcl-G
polypeptides so mutated as to be incapable of normal
activity. Therefore, the transgenic non-human mammals do
not express native Bcl-G or have reduced expression of
native Bcl-G. The present invention also provides
transgenic non-human mammals having a genome comprising
antisense nucleic acids complementary to nucleic acids
encoding Bcl-G polypeptides, placed so as to be
transcribed into antisense mRNA complementary to mRNA
encoding Bcl-G polypeptides, which hybridizes to the mRNA
and, thereby, reduces the translation thereof. The
nucleic acid can additionally comprise an inducible
promoter and/or tissue specific regulatory elements, so

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that expression can be induced, or restricted to specific cell types.

Animal model systems useful for elucidating the physiological and behavioral roles of Bcl-G polypeptides are also provided, and are produced by creating transgenic animals in which the expression of the Bcl-G polypeptide is altered using a variety of techniques. Examples of such techniques include the insertion of normal or mutant versions of nucleic acids encoding an Bcl-G polypeptide by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos to produce a transgenic animal, see, for example, Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual (Cold Spring Harbor Laboratory, (1986)). Transgenic animal model systems are useful for *in vivo* screening of compounds for identification of specific ligands, such as agonists or antagonists, which activate or inhibit a biological activity.

Also contemplated herein, is the use of homologous recombination of mutant or normal versions of Bcl-G genes with the native gene locus in transgenic animals, to alter the regulation of expression or the structure of Bcl-G polypeptides by replacing the endogeneous gene with a recombinant or mutated Bcl-G gene. Methods for producing a transgenic non-human mammal including a gene knock-out non-human mammal, are well known to those skilled in the art (see, Capecchi et al., Science 244:1288 (1989); Zimmer et al., Nature 338:150 (1989); Shastry, Experentia, 51:1028-1039 (1995); Shastry, Mol. Cell. Biochem., 181:163-179 (1998); and U.S. Patent No. 5,616,491, issued April 1, 1997, No. 5,750,826, issued May 12, 1998, and No. 5,981,830, issued

November 9, 1999).

        Invention nucleic acids, oligonucleotides,
including antisense, vectors containing invention nucleic
acids, transformed host cells, polypeptides and
5   combinations thereof, as well as antibodies of the
present invention, can be used to screen compounds to
determine whether a compound functions as a potential
agonist or antagonist of invention polypeptides. These
screening assays provide information regarding the
10   function and activity of invention polypeptides, which
can lead to the identification and design of compounds
that are capable of specific interaction with one or more
types of polypeptides, peptides or proteins.

15   Thus, the invention provides methods for
identifying compounds which bind to Bcl-G polypeptides.
The invention proteins can be employed in a competitive
binding assay. Such an assay can accommodate the rapid
screening of a large number of compounds to determine
20   which compounds, if any, are capable of binding to Bcl-G
polypeptides. Subsequently, more detailed assays can be
carried out with those compounds found to bind, to
further determine whether such compounds act as
modulators, agonists or antagonists of invention Bcl-G
25   polypeptides. Compounds that bind to and/or modulate
invention Bcl-G polypeptides can be used to treat a
variety of pathologies mediated by invention Bcl-G
polypeptides.

        Various binding assays to identify cellular
30   proteins that interact with protein binding domains are
known in the art and include, for example, yeast
two-hybrid screening assays (see, for example, U.S.
Patent Nos. 5,283,173, 5,468,614 and 5,667,973; Ausubel

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et al., *supra*, 1999; Luban et al., <u>Curr. Opin.</u>
<u>Biotechnol</u>. 6:59-64 (1995)) and affinity column
chromatography methods using cellular extracts. By
synthesizing or expressing polypeptide fragments
5   containing various Bcl-G sequences or deletions, the Bcl-
G binding interface can be readily identified.

        In another embodiment of the invention, there
is provided a bioassay for identifying compounds which
modulate the activity of invention Bcl-G polypeptides.
10   According to this method, invention polypeptides are
contacted with an "unknown" or test substance, for
example, in the presence of a reporter gene construct
responsive to a Bcl-G signaling pathway, the activity of
the polypeptide is monitored subsequent to the contact
15   with the "unknown" or test substance, and those
substances which cause the reporter gene construct to be
expressed are identified as functional ligands for Bcl-G
polypeptides. Such reporter gene assays and systems are
well known to those skilled in the art (Ausubel et al.,
20   supra, 1999). In addition, a reporter gene constrict can
be generated using the promoter region of Bcl-G and
screened for compounds that increase or decrease Bcl-G
gene promoter activity. Such compounds can also be used
to alter Bcl-G expression.

25   In accordance with another embodiment of the
present invention, transformed host cells that
recombinantly express invention polypeptides can be
contacted with a test compound, and the modulating
effect(s) thereof can then be evaluated by comparing the
30   Bcl-G-mediated response, for example, via reporter gene
expression in the presence and absence of test compound,
or by comparing the response of test cells or control
cells, to the presence of the compound.

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As used herein, a compound or a signal that
"modulates the activity" of invention polypeptides refers
to a compound or a signal that alters the activity of
Bcl-G polypeptides so that the activity of the invention

5   polypeptide is different in the presence of the compound
or signal than in the absence of the compound or signal.
In particular, such compounds or signals include agonists
and antagonists. An agonist encompasses a compound or a
signal that activates Bcl-G protein expression or

10   biological activity. Alternatively, an antagonist
includes a compound or signal that interferes with Bcl-G
expression or biological activity. Typically, the effect
of an antagonist is observed as a blocking of agonist-
induced protein activation. Antagonists include

15   competitive and non-competitive antagonists.

Assays to identify compounds that modulate Bcl-
G polypeptide expression can involve detecting a change
in Bcl-G polypeptide abundance in response to contacting
the cell with a compound that modulates Bcl-G activity.

20   Assays for detecting changes in polypeptide expression
include, for example, immunoassays with Bcl-G-specific
Bcl-G antibodies, such as immunoblotting,
immunofluorescence, immunohistochemistry and
immunoprecipitation assays, as described above.

25   As understood by those of skill in the art,
assay methods for identifying compounds that modulate
Bcl-G activity generally require comparison to a control.
One type of a "control" is a cell or culture that is
treated substantially the same as the test cell or test

30   culture exposed to the compound, with the distinction
that the "control" cell or culture is not exposed to the
compound. Another type of "control" cell or culture can
be a cell or culture that is identical to the test cells,

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with the exception that the "control" cells or culture do
not express a Bcl-G polypeptide. Accordingly, the
response of the transfected cell to a compound is
compared to the response, or lack thereof, of the
5   "control" cell or culture to the same compound under the
same reaction conditions.

Methods for producing pluralities of compounds
to use in screening for compounds that modulate the
activity of a Bcl-G polypeptide, including chemical or
10   biological molecules such as simple or complex organic
molecules, metal-containing compounds, carbohydrates,
peptides, proteins, peptidomimetics, glycoproteins,
lipoproteins, nucleic acids, antibodies, and the like,
are well known in the art and are described, for example,
15   in Huse, U.S. Patent No. 5,264,563; Francis et al., Curr.
Opin. Chem. Biol. 2:422-428 (1998); Tietze et al., Curr.
Biol., 2:363-371 (1998); Sofia, Mol. Divers. 3:75-94
(1998); Eichler et al., Med. Res. Rev. 15:481-496 (1995);
and the like. Libraries containing large numbers of
20   natural and synthetic compounds also can be obtained from
commercial sources. Combinatorial libraries of molecules
can be prepared using well known combinatorial chemistry
methods (Gordon et al., J. Med. Chem. 37: 1233-1251
(1994); Gordon et al., J. Med. Chem. 37: 1385-1401
25   (1994); Gordon et al., Acc. Chem. Res. 29:144-154 (1996);
Wilson and Czarnik, eds., Combinatorial Chemistry:
Synthesis and Application, John Wiley & Sons, New York
(1997)).

Compounds that modulate Bcl-G activity can be
30   screened by the methods disclosed herein to identify
compounds that modulate any biological activity or
function of Bcl-G. For example, compounds can be
identified that alter the interaction of Bcl-G with Bcl-2

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family members. Additionally, compounds can be
identified that modulate ion channel activity associated
with Bcl-G. The formation of ion channels by Bcl-2
family members is one mechanism of inducing apoptosis in
5   cells (Reed, *supra*, 1998). Therefore, compounds that
modulate ion channel activity of Bcl-G can be used to
alter apoptosis, thereby increasing or decreasing
apoptotic activity of Bcl-G.

Another assay for screening of compounds that
10   modulates the activity of Bcl-G is based on altering the
phenotype of yeast by expressing Bcl-G. For example,
expression of Bax in yeast confers a lethal phenotype
(Matsuyama et al., Mol. Cell. 1:327-336 (1998)). A yeast
that expresses Bcl-G can have a similar phenotype as Bax
15   since the biological activity of Bcl-G is similar to Bax
(Example III). Accordingly, a yeast strain expressing
Bcl-G that confers a lethal phenotype can be screened for
compounds that prevent cell death. In one embodiment,
expression of Bcl-G can be inducible (Tao et al., J.
20   Biol. Chem. 273:23704-23708 (1998), and the compounds can
be screened when Bcl-G expression is induced. Bcl-G can
also be co-expressed in yeast with other Bcl-2 family
members having anti-apoptotic activity such as Bcl-2 or
Bcl-X_L. For example, co-expression of Bax with Bcl-2 or
25   Bcl-X_L suppressed the lethal activity of Bax (Tao et al.,
supra, 1998). Similarly, co-expression of Bcl-G with an
anti-apoptotic Bcl-2 family member such as Bcl-2 or Bcl-X_L
can be used to screen for compounds that antagonize the
activity of the anti-apoptotic Bcl-2 family members and
30   restore a lethal phenotype. Such compounds can function
to inhibit binding of Bcl-G to anti-apoptotic Bcl-2
family members such as Bcl-2 or Bcl-X_L.

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In yet another embodiment of the present
invention, the activation of Bcl-G polypeptides can be
modulated by contacting the polypeptides with an
effective amount of at least one compound identified by
5   the assays described herein. The invention also provides
a method of identifying an effective agent that alters
the association of a Bcl-G with a Bcl-G associated
polypeptide (BAP). The method includes the steps of
contacting the Bcl-G and the BAP polypeptide, under
10   conditions that allow said Bcl-G and BAP polypeptide to
associate, with a compound; and detecting the altered
association of the Bcl-G and BAP polypeptide, thereby
identifying a compound that is an effective agent for
altering the association of Bcl-G with BAP. The compound
15   can be, for example, a drug or polypeptide. A BAP can
be, for example, Bcl-2 family member such as Bcl-2 or
$Bcl-X_L$.

As disclosed herein, Bcl-G is a new member of
the Bcl-2 family that has pro-apoptotic activity (see
20   Example III). Therefore, modulation of Bcl-G activity
can be advantageously used to modulate the level of
apoptosis in a cell. For example, increasing the
activity of Bcl-G can be used to promote apoptosis in a
cell. Bcl-G activity can be increased, for example, by
25   increasing the level of a Bcl-G polypeptide or functional
fragment thereof. Increased levels of a Bcl-G
polypeptide can be accomplished, for example, by
delivering to a cell a nucleic acid encoding Bcl-G and
expressing a Bcl-G polypeptide recombinantly or by
30   delivering a Bcl-G polypeptide or functional fragment
thereof directly to a target by the methods disclosed
herein. Additionally, Bcl-G activity can be increased by
using a modulatory agent that functions as an agonist.
Promoting apoptosis by increasing Bcl-G activity or

expression is useful, for example, in therapeutic applications such as the treatment of cancer.

As disclosed herein, decreases or loss of Bcl-G is associated with approximately 50% of prostate cancers, approximately 30% of ovarian cancers and approximately 30 % of leukemias. Bcl-G can function as a tumor suppressor. Therefore, methods of administering a Bcl-G polypeptide either directly or using an encoded nucleic acid can be used to treat a cancer. Furthermore, many chemotherapeutic agents function through increasing apoptosis. Therefore, the invention additionally provides a method to enhance a chemotherapy by increasing Bcl-G activity or expression. Administering Bcl-G can thus be used to enhance the effect of standard chemotherapeutic agents.

Alternatively, modulation of Bcl-G activity can be advantageously used to decrease Bcl-G activity to decrease apoptosis. For example, Bcl-G activity or expression can be decreased by administering an anti-sense Bcl-G nucleic acid. In addition, an antagonist of Bcl-G activity can be identified by the methods disclosed herein and used to decrease Bcl-G activity. Decreasing Bcl-G activity can be used to inhibit apoptosis. Inhibiting apoptosis can be useful, for example, to treat disease ischemic. For example, decreasing Bcl-G activity with anti-sense nucleic acids or small molecule compounds can be used to treate stroke, heart attack, autoimmunity, trauma, neuron cell death, and inflammatory diseases, including Crohn's disease. For example, Bcl-G was identified in Crohn's disease patients (see Example I).

The invention further provides a method for modulating an activity mediated by a Bcl-G polypeptide by

contacting the Bcl-G polypeptide with an effective, modulating amount of an agent that modulates Bcl-G activity. The Bcl-G activity can be, for example, apoptosis-inducing activity, binding to Bcl-2, or tumor

5   suppresor activity. The invention additionally provides a method of modulating the level of apoptosis in a cell. The method includes the steps of introducing a nucleic acid molecule encoding a Bcl-G into the cell; and expressing the Bcl-G in the cell, wherein the expression

10   of the Bcl-G modulates apoptosis in the cell.

The invention further provides a method of modulating the level of apoptosis in a cell by contacting the cell with a compound that effectively alters the association of Bcl-G with a Bcl-G-associated-protein in

15   the cell, or that effectively alters the activity of a Bcl-G in the cell. Additionally provided by the invention is a method of modulating interactions between Bcl-G and Bcl-2 by contacting a Bcl-G polypeptide with the agent that inhibits or alters interactions between

20   Bcl-G and Bcl-2.

As disclosed herein, Bcl-G is located on chromosome 12 in a region deleted in various cancers, including leukemia, prostate and ovarian cancer (Example IV). Therefore, methods using Bcl-G nucleic acids or

25   antibodies can be used as a diagnostic for predisposition or progression of cancer, for example, leukemia, prostate or ovarian cancer. Changes in Bcl-G expression or activity can be correlated with patient survival or response to therapy, and a correlation can be used to

30   monitor cancer progression or response to therapy.

The invention further provides a method of diagnosing a pathology characterized by an increased or

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decreased level of a Bcl-G in a subject. The method
includes the steps of (a) obtaining a test sample from
the subject; (b) contacting the sample with an agent that
can bind the Bcl-G under suitable conditions, wherein the
5   conditions allow specific binding of the agent to the
Bcl-G; and (c) comparing the amount of the specific
binding in the test sample with the amount of specific
binding in a control sample, wherein an increased or
decreased amount of the specific binding in the test
10   sample as compared to the control sample is diagnostic of
a pathology. The agent can be, for example, an anti-Bcl-
G antibody, a Bcl-G-associated-protein (BAP), or a Bcl-G
nucleic acid.

The invention also provides a method of
15   diagnosing cancer or monitoring cancer therapy by
contacting a test sample from a patient with a Bcl-G-
specific antibody. The invention additionally provides a
method of assessing prognosis of patients with cancer
comprising contacting a test sample from a patient with a
20   Bcl-G-specific antibody.

The invention additionally provides a method of
diagnosing cancer or monitoring cancer therapy by
contacting a test sample from a patient with a Bcl-G
oligonucleotide. The invention further provides a method
25   of assessing prognosis of patients with cancer by
contacting a test sample from a patient with a Bcl-G
oligonucleotide.

The methods of the invention for diagnosing
cancer or monitoring cancer therapy using a Bcl-G-
30   specific antibody or Bcl-G oligonucleotide or nucleic
acid can be used, for example, to segregate patients into
a high risk group or a low risk group for predicting risk

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of metastasis or risk of failure to respond to therapy.
Therefore, the methods of the invention can be
advantageously used to determine the risk of metastasis
in a cancer patient or as a prognostic indicator of

5   survival in a cancer patient. One of ordinary skill in
the art would appreciate that the prognostic indicators
of survival for cancer patients suffering from stage I
cancer can be different from those for cancer patients
suffering from stage IV cancer. For example, prognosis

10 for stage I cancer patients can be oriented toward the
likelihood of continued growth and/or metastasis of the
cancer, whereas prognosis for stage IV cancer patients
can be oriented toward the likely effectiveness of
therapeutic methods for treating the cancer.

15 Accordingly, the methods of the invention directed to
measuring the level of or determining the presence of a
Bcl-G polypeptide or encoding nucleic acid can be used
advantageously as a prognostic indicator for the presence
or progression of a cancer or response to therapy.

20   In accordance with another embodiment of the
present invention, there are provided diagnostic systems,
preferably in kit form, comprising at least one invention
nucleic acid or antibody in a suitable packaging
material. The diagnostic kits containing nucleic acids

25 are derived from the Bcl-G-encoding nucleic acids
described herein. In one embodiment, for example, the
diagnostic nucleic acids are derived from any of SEQ ID
NOS:1, 3 or 41 and can be oligonucleotides of the
invention. Invention diagnostic systems are useful for

30 assaying for the presence or absence of nucleic acid
encoding Bcl-G in either genomic DNA or mRNA.

        A suitable diagnostic system includes at least
one invention nucleic acid or antibody, as a separately

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packaged chemical reagent(s) in an amount sufficient for at least one assay. For a diagnostic kit containing nucleic acid of the invention, the kit will generally contain two or more nucleic acids. When the diagnostic kit is to be used in PCR, the kit will contain at least two oligonucleotides that can serve as primers for PCR. Those of skill in the art can readily incorporate invention nucleic probes and/or primers or invention antibodies into kit form in combination with appropriate buffers and solutions for the practice of the invention methods as described herein. A kit containing a Bcl-G antibody can contain a reaction cocktail that provides the proper conditions for performing an assay, for example, an ELISA or other immunoassay, for determining the level of expression of a Bcl-G polypeptide in a sample, and can contain control samples that contain known amounts of a Bcl-G polypeptide and, if desired, a second antibody specific for the anti-Bcl-G antibody.

The contents of the kit of the invention, for example, Bcl-G nucleic acids or antibodies, are contained in packaging material, preferably to provide a sterile, contaminant-free environment. In addition, the packaging material contains instructions indicating how the materials within the kit can be employed both to detect the presence or absence of a particular Bcl-G sequence or Bcl-G polypeptide or to diagnose the presence of, or a predisposition for a condition associated with the presence or absence of Bcl-G such as cancer. The instructions for use typically include a tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

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It is understood that modifications which do
not substantially affect the activity of the various
embodiments of this invention are also provided within
the definition of the invention provided herein.
5   Accordingly, the following examples are intended to
illustrate but not limit the present invention.

EXAMPLE I

Molecular Cloning of Bcl-G

This example describes the cloning of Bcl-G, a
10   homologue of Bcl-2.

To clone the full length Bcl-G gene,
oligonucleotide primers were designed based a short EST
(GenBank Accession No. AW000827) from colonic mucosa of 3
patients with Crohn's disease found by searching a
15   database for sequences similar to the BH2 and BH3 domains
of Bcl-2 family proteins. The primers used were Primer 1
(5'GTACTTGGTGCCAAAGCCCAGG-3'; SEQ ID NO:7) and Primer 2
(5'-GACATGATGTCTGGTGTAGTAGGCGAGG-3'; SEQ DI NO:8). The
full length Bcl-G cDNA was cloned using SMART™RACE cDNA
20   Amplification Kit (Clontech; Palo Alto CA) from human
placental total RNA (Clontech) as template. The 5'-RACE
products were sequenced with an automated sequencer.

Briefly, for cloning of Bcl-G cDNAs, TBLAST
searches of the public databases using human Bcl-2 as a
25   query sequence revealed a short EST (GenBank AW000827)
from colonic mucosa of 3 patients with Crohn's disease
which contains an open reading frame (ORF) encoding
sequences similar to the BH2 domain of Bcl-2 family
proteins. An oligonucleotide primer
30   (5'-GTACTTGGTGCCAAAGCCCAGG-3'; SEQ ID NO:7) was designed
complementary to the EST sequence and used for 5'-RACE,

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employing the SMART™ RACE cDNA Amplification Kit
(Clontech; Palo Alto CA) and human placental total RNA as
template. The 5'-RACE products were subcloned into
pCR2.1-TOPO vector using the TOPO™ TA Cloning kit
5   (Invitrogen; Carlsbad CA), and their DNA sequence
determined, revealing a complete open reading frame
(ORF), with start codon within a favorable Kozak sequence
context, preceded by a 5' -untranslated region (UTR)
containing stop codons in all three reading-frames
10   (submitted to Genbank). Two additional EST clones,
AI478889 and AI240211, were identified by BLAST searches,
corresponding to overlapping partial Bcl-G cDNAs which
contained the 3' -UTR.

A short EST was identified during searches of
15   the public databases, which when conceptually translated
revealed a polypeptide sequence with similarity to the
BH2 domain of Bcl-2 family proteins. Full-length cDNAs
were obtained, revealing two potential transcripts
containing open reading frames (ORF) for proteins of 327
20   and 252 amino-acids, respectively, which were termed
Bcl-G$_L$ and Bcl-G$_S$ (Figure 5A). The predicted Bcl-G$_L$ and
Bcl-G$_S$ proteins are identical for the first 226 amino
acids, then diverge thereafter. Comparison of the
predicted amino acid sequences of Bcl-G$_L$ and Bcl-G$_S$ with
25   Bcl-2 family proteins revealed the presence of a
candidate BH3 domain (SEQ ID NO:9) in both Bcl-G$_L$ and Bcl-
G$_S$ (Figure 5A,B), and the presence of a BH2 domain (SEQ ID
NO:18) in Bcl-G$_L$ but not in Bcl-G$_S$ (Figure 5A, C).

Invention Bcl-G was found to exist in two
30   forms, a long form, designated Bcl-G$_L$, and a shorter form,
designated Bcl-G$_S$. The nucleotide sequence of Bcl-G$_L$ is
shown in Figure 1 (SEQ ID NO:1). The nucleotide sequence
of the coding region of Bcl-G$_L$ cDNA and the encoded amino

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acid sequence (SEQ ID NO:3) are shown in Figure 2. Bcl-G$_L$
was initially identified to contain a core BH3 domain
(^{216}LKYSGDQLE^{224}; SEQ ID NO:5) and a core BH2 domain
(^{307}PWIQQHGGWE^{316}; SEQ ID NO:6).

5   The shorter form of Bcl-G, Bcl-G$_S$, is an
apparent alternative splicing product of Bcl-G mRNA. The
nucleotide sequence of Bcl-G$_S$ is shown in Figure 3 (SEQ ID
NO:3). The nucleotide sequence of the coding region of
Bcl-G$_S$ cDNA and the encoded amino acid sequence (SEQ ID
10   NO:4) are shown in Figure 2. Bcl-G$_S$ contains only the BH3
domain (^{216}LKYSGDQLE^{224}).

         These results demonstrate that a new member of
the Bcl-2 family, Bcl-G, is expressed in human placenta
and in the colonic mucosa of patients with Crohn's
15   disease. Bcl-G exists in two forms, a long form,
designated Bcl-G$_L$, which contains a BH2 and BH3 domain,
and a short form, designated Bcl-G$_S$, which contains only a
BH3 domain.

                         EXAMPLE II
20   **Mapping of Bcl-G to Chromosome 12p12.3**

         This experiment describes chromosomal mapping
of human Bcl-G.

         To map the chromosomal location of Bcl-G, a
search of the GenBank database was performed using BLAST
25   (Altschul et al., J. Mol. Biol. 215:403-410 (1990);
Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997).
A 190858 bp human 12p12 BAC chromosome sequence
RPCI11-267J23 (GenBank accession no. AC007537) was found
to contain the full length Bcl-G gene. The BAC also
30   contains the LRP6 gene (exon 1 starts at 89963 bp). A

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600 kb region between 12p12.3 to 12p13.1, flanked by
D12S358 and ETV6/exon8, was previously defined to be
frequently deleted in childhood acute lymphoblastic
leukemia (ALL) and other solid tumor cells (Baens et al.,
5   (1999) Genomics 56:40-50 (1999); Hatta et al., Br. J.
Cancer 75:1256-1262 (1997); Kibel et al., Cancer Res.
58:5652-5655 (1998); Baccichet et al., Br. J. Haematol.
99:107-114 (1997); Aissani et al., Leuk. Lymphoma
34:231-239). The loss of the region containing Bcl-G
10   occurs in approximately 50% of prostate cancers, 30% of
ovarian cancers, and 30% of leukemias.

The LRP6 gene is located in the region between
12p12.3 to 12p13.1. Using LRP6 as a marker for
orientation, Bcl-G was located in this region. Exon 1 of
15   Bcl-G starts at 40674 bp in the BAC and was deduced from
novel DNA sequence data obtained from 5' RACE-based
amplification of the full-length Bcl-G cDNA. The genomic
structure of the Bcl-G gene is shown in Figure 5. Bcl-G$_L$
has 6 exons, with the first codon non-coding, spreading
20   across a 30 kb region in chromosome 12. Bcl-G$_S$ also has 6
exons, but a 153 bps sequence is inserted in front of
exon 5 and contains a stop codon. The BH3 domain is
located in exon 4 of both Bcl-G$_L$ and Bcl-G$_S$. The BH2
domain is located in exon 5 of Bcl-G$_L$. The Bcl-G$_L$ and
25   Bcl-G$_S$ cDNAs can be accounted for by an alternative mRNA
splicing mechanism in which different splice acceptor
sites associated with exon 5 are employed, resulting in a
change in the distal reading-frame (Figure 5D).

The chromosomal mapping of Bcl-G to chromosome
30   12p12.3 is shown in Figure 6. Bcl-G is located in a 600
kb region that has been previously determined to be
frequently deleted in childhood ALL and other solid
tumors (Baens et al., supra, 1999). Therefore, Bcl-G is

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located in a region deleted in ALL and can function as a
tumor suppressor or as a marker for tumor suppressor
activity.

Example III

5   **Expression of Bcl-G**

          This example describes the expression of Bcl-G.

          For generation of plasmids, cDNAs containing
the ORFs of Bcl-G$_L$ and Bcl-G$_S$ without additional flanking
sequences were generated by PCR using human placental
10   cDNA as a template and the following primers:
5'-GGCTCGAGCGATGTGTAGCACCAGTGGGTGTGACC-3' (SEQ ID NO:27),
sense for both Bcl-G$_L$ and Bcl-G$_S$;
5'-CCAAGCTTTAAGTCTACTTCTTCATGTGATATCCC-3' (SEQ ID NO:28),
antisense for Bcl-G$_L$; and
15   5'-CCAAGCTTTAAAATGCAGGCCATCAAACC-3' (SEQ ID NO:29),
antisense for Bcl-G$_S$. The resulting PCR products were
digested with restriction endonucleases and subcloned
into the Xho I and Hind III sites of pEGFP-C1 (Clontech).
A mutant of Bcl-G$_S$ lacking the BH3 domain was created by a
20   two-step PCR method, using the following primers:
primer1, 5'- GGCTCGAGCGATGTGTAGCACCAGTGGGTGTGACC-3' (SEQ
ID NO:30); primer2,
5'-CCGGATCCGGCTAGTATTTGTTCTTCTTCATCTTTC-3' (SEQ ID
NO:31); primer3, 5'-CCGGATCCGACACTGCCTTCATCCCCATTCCC-3'
25   (SEQ ID NO:32); and primer4,
5'-CCAAGCTTTAAAATGCAGGCCATCAAACC-3' (SEQ ID NO:33). The
resulting PCR product was digested with XhoI / BamHI or
with BamHI / HindIII respectively, and ligated into
pEGFP-C1. Site-directed mutagenesis of Bcl-G$_S$ was
30   performed to generate a L216E substitution mutation using
the QuikChange™ Site-Directed Mutagenesis Kit
(Stratagene) following manufacturer's procedure, with

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pEGFP-C1/Bcl-G$_S$ plasmid as DNA template, and the mutagenic primers: 5'-GCCAAAATTGTTGAGCTGGAGAAATATTCAGGAGATCAGTTGG-3' (SEQ ID NO:34) and 5'-CCAACTGATCTCCTGAATATTTCTCCAGCTCAACAATTTTGGC-3' (SEQ ID NO:35).

For measurements of Bcl-G mRNAs, Bcl-G mRNAs were detected by either Northern blotting or Reverse-Transcriptase-Polymerase Chain Reaction (RT-PCR). For RT-PCR, multiple-tissue cDNA panels (Clontech) containing first-strand cDNA generated from 16 different tissues were employed. PCR was performed according to the manufacturer's protocol with following primers: (a) 5' primer for both Bcl-G$_S$ and Bcl-G$_L$, corresponding to exon 3, 5'-CTGAGGGTCTCTCCTTCCAGCTCCAAGG-3' (SEQ ID NO:36); (b) 3' primer for Bcl-G$_L$, corresponding to exon 5, 5'-GGCCGTGACGTCTATTACAAGGGCAGCC-3' (SEQ ID NO:37); and 3' primer for Bcl-G$_S$, corresponding to an alternatively spliced segment of exon 5, 5'-CAAGGGAATGGGGATGAAGGCAGTGTC-3' (SEQ ID NO:38). Human G3PDH expression was examined by PCR with the following primers: 5'-TGAAGGTCGGAGTCAACGGATTTGGT-3' (SEQ ID NO:39)(sense); and 5'-CATGTGGGCCATGAGGTCCACCAC- 3' (SEQ ID NO:40)(antisense).

For tissue-specific expression of Bcl-G$_L$ and Bcl-G$_S$ mRNAs, Northern blotting demonstrated the presence of ~2 kbp Bcl-G transcripts in several normal human tissues, but failed to resolve the mRNAs encoding Bcl-G$_L$ and Bcl-G$_S$. RT-PCR assays were therefore designed using primers specific for Bcl-G$_L$ and Bcl-G$_S$ sequences associated with exon 5. Bcl-G$_L$ mRNA was clearly detected in lung, pancreas, prostate and testis, with lower levels present in some other tissues (Figure 7). In contrast, Bcl-G$_S$ mRNA was uniquely expressed in testis. RT-PCR

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amplification of a control mRNA, G3PDH, demonstrated
loading of nearly equivalent amounts of mRNA from each
tissue. The amplified bands corresponding to Bcl-G_L and
Bcl-G_S were excised and sequenced, confirming the validity
5   of the RT-PCR strategy.

EXAMPLE IV

Induction of Cell Death by Bcl-G_S

       This experiment describes the induction of cell
death by Bcl-G_S in transfected PC-3 cells.

10   For cell culture, transfections, and apoptosis
assay, 293T and Cos-7 cells were cultured in DMEM high
glucose media (Irvine Scientific, Santa Ana, CA)
containing 10% fetal bovine serum (FBS). PC-3 cells were
cultured with RPMI 1640 media containing 10% FBS.
15   Transfection of cells was performed using SuperFect
(Qiagen, Chatsworth, CA). Both floating and adherent
cells (after trypsinization) were collected 24 hrs after
transfection, fixed, and stained using 4',6-diamidine-2'-
phenylindole dihydrochloride (DAPI) for assessing
20   apoptosis based on nuclear fragmentation and chromatin
condensation (Xu & Reed, Mol. Cell, 1:337-346 (1998);
Zhang et al., Proc. Natl. Acad. Sci. (USA), 97:2597-2602
(2000)).

       To characterize a biological function of Bcl-G,
25   a Bcl-G_S construct was generated by cloning Bcl-G_S cDNA
into pcDNA3.1/Myc/His expression vector (Invitrogen;
Carlsbad CA) at the Xho I / Hind III sites. The
authenticity of the construct was confirmed by DNA
sequencing.

For transfection experiments, various vectors were transfected into PC-3 cells: control vector pcDNA3.1/Myc/His; pcDNA3.1/Myc/His/Bcl-G$_s$ expressing Bcl-G$_s$; pRC/CMV/Bcl-2 expressing Bcl-2; and pRC/CMV/Bax expressing Bax. The vectors were transfected as follows: pcDNA3.1/Myc/His alone; pcDNA3.1/Myc/His/Bcl-G$_s$ alone; pcDNA3.1/Myc/His/Bcl-G$_s$ + pRC/CMV/Bcl-2; pRC/CMV/Bax alone; or pRC/CMV/Bax + pRC/CMV/Bcl-2. One μg of each vector was combined with 0.2 μg pEGFP-N2 (Clontech), and the vectors were transiently transfected into PC-3 prostate cancer cells using SuperFect reagent (QIAGEN; Valencia CA), following the instructions of the manufacturer. At 24 hours after transfection, cells were examined under a fluorescent microscope. About 100 green fluorescent protein (GFP) positive (green color) cells were counted for each transfection. Cells that were detached with membrane blebbing and/or apoptotic bodies were recorded as dead cells. Results were averaged from three separate transfections.

As shown in Figure 8, Bcl-G$_s$ induces cell death in PC-3 cells (compare "control" to "Bcl-G$_s$"). The induction of cell death by Bcl-G$_s$ was similar to Bax, which was used as a positive control based on its known pro-apoptotic activity (compare "Bcl-G$_s$" to "Bax"). The induction of cell death by Bcl-G$_s$ was completely inhibited when co-transfected with the anti-apoptotic Bcl-2 (see "Bcl-G$_s$ + Bcl-2"). The inhibition of Bcl-G$_s$-induced cell death by Bcl-2 was similar to that seen with Bax (see "Bax + Bcl-2").

To assess the effects of Bcl-G$_L$ and further assess the effects of Bcl-G$_s$ on apoptosis, various cell lines, including Cos7, HEK293T, and PC3, were transiently transfected with plasmids encoding Bcl-G$_L$ or Bcl-G$_s$. For

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most experiments, Bcl-G$_L$ and Bcl-G$_S$ were expressed as GFP-
fusions so that successfully transfected cells could be
conveniently identified (Figure 9A), but similar results
were obtained when Flag-epitope tags were employed

5   instead.   Over-expression of the shorter Bcl-G$_S$ protein
reproducibly induced striking increases in the percentage
of cells undergoing apoptosis, as determined by DAPI
staining (Figure 9) and other methods.   In contrast, Bcl-
G$_L$ was more variable and less efficient at inducing

10   apoptosis in these transient transfection assays.
Immunoblot analysis of lysates from transfected cells
demonstrated that the less potent effects of Bcl-G$_L$ could
not be accounted for by lower levels of protein
production (Figure 9A).   Indeed, Bcl-G$_L$ protein

15   accumulated to levels ~10-fold higher in cells compared
to Bcl-G$_S$, suggesting that Bcl-G$_S$ is a far more potent
apoptosis-inducer.   Analysis of the same blots with an
anti-tubulin antibody confirm loading of essentially
equivalent amounts of total protein for each sample, thus

20   validating the results.   In additional transfection
experiments, Bcl-G$_L$ failed to demonstrate cytoprotective
activity in side by side comparisons with Bcl-2 and
Bcl-X$_L$.

EXAMPLE V

25   **The BH3 Domain of Bcl-G$_S$ is Required for its Pro-apoptotic
Activity**

The Bcl-G$_S$ protein contains a BH3 domain, but
lacks other regions of homology with Bcl-2 family
proteins.   Structural studies indicate that BH3 domains

30   represent amphipathic α-helices, in which the hydrophobic
surface of the α-helices of apoptosis-inducing BH3
peptides bind to a pocket on survival proteins such as
Bcl-X$_L$ (Sattler et al., Science, 275:983-986 (1997)).

Therefore, the apoptosis-inducing activity of the
wild-type $Bcl-G_S$ protein was compared with mutants lacking
the BH3 domain (ΔBH3) or in which leucine 216 within the
BH3 domain of $Bcl-G_S$ was chosen for mutation to charged

5   glutamic acid, based on comparisons with previously
described BH3 mutagenesis experiments demonstrating a
critical requirement for the analogous leucine in other
pro-apoptotic Bcl-2 family proteins (Wang et al., Mol.
Cell. Biol., 18:6083-6089 (1998); Kelekar et al., Mol.

10   Cell. Biol., 17:7040-7046 (1997)).

       Wild-type $Bcl-G_S$ potently induced apoptosis when
overexpressed in Cos-7, PC3, HEK293T and other cell
lines, whereas $Bcl-G_S$ (ΔBH3) and $Bcl-G_S$ (L216E) did not
(see Figure 3B). Immunblot analysis confirmed production

15   of the $Bcl-G_S$ (ΔBH3) and $Bcl-G_S$ (L216E) proteins at levels
exceeding the amounts of wild-type $Bcl-G_S$ protein.
Therefore, the BH3 domain of $Bcl-G_S$ is critical for its
pro-apoptotic activity.

EXAMPLE VI

20   **$Bcl-G_S$ Associates with $Bcl-X_L$ in a BH3-dependent Manner**

       The pro-apoptotic activity of "BH3-only"
members of the Bcl-2 family depends on their ability to
dimerize with and suppress the activity of survival
proteins such as $Bcl-X_L$ (reviewed in Kelekar & Thompson,

25   Trends Cell Biol., 8:324-330 (1998)). It was, therefore,
determined whether $Bcl-G_L$ and $Bcl-G_S$ are capable of
associating with other Bcl-2 family proteins by co-
immunoprecipitation assays.

       For co-immunoprecipitations and immunoblotting,

30   immunoblotting was performed as described previously (Xu
and Reed, supra., (1998); Zhang et al., supra., (2000)).

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For co-immunoprecipitations, cells were cultured in 50 mM benzocarbonyl Valine Alanine Aspartate fluoromethyl-ketone (zVAD-fmk) to prevent apoptosis. Cells were suspended in lysis buffer (50 mM Tris-HCl, pH7.4; 150 mM

5   NaCl; 20 mM EDTA; 50 mM NaF; 0.5% NP-40; 0.1 mM Na_3VO_4; 20 µg/ml Leupeptin; 20 µg/ml Aprotinin; 1 mM dithiothreitol (DTT); and 1 mM phenylmethylsulfonylfluoride (PMSF). Lysates (0.2 ml diluted into 1 ml final volume of lysis buffer) were cleared by incubation with 15 µl of protein

10   G-Sepharose 4B (Zymed; South San Francisco CA) and then incubated with 15 µl of polyclonal anti-GFP antibody (Santa Cruz; Santa Cruz CA) and 15 µl of protein G at 4°C overnight. Beads were then washed 4 times with 1.5 mls lysis buffer before boiling in Laemmli sample buffer and

15   performing SDS-PAGE/immunoblotting.

       Bcl-G_S association with the survival proteins Bcl-X_L and Bcl-2 was readily detected by co-immunoprecipitation using lysates from transiently transfected cells, whereas no association with pro-

20   apoptotic proteins Bax, Bak, Bid or Bad was observed (Figure 10A). Interaction of Bcl-G_S with Bcl-2 and Bcl-X_L, but not with Bax or Bak, was also confirmed by yeast two-hybrid assays. In contrast, association of the longer Bcl-G_L protein with Bcl-2 or Bcl-X_L was not easily

25   detected by co-immunoprecipitation assays (Figure 10A). With much longer x-ray film exposure times, however, small amounts of Bcl-X_L were observed in association with Bcl-G_L immunocomplexes, suggesting either low affinity binding of Bcl-G_L to Bcl-X_L or implying that only a small

30   portion of total Bcl-G_L proteins are competent to bind Bcl-X_L. The interaction of Bcl-G_S with Bcl-X_L was BH3-dependent, as determined by comparisons of wild-type Bcl-G_S with the Bcl-G_S (ΔBH3) and Bcl-G_S (L216E) proteins (Figures 10B, C). Thus, the pro-apoptotic activity of

Bcl-G$_S$ correlates with it ability to bind Bcl-X$_L$.

EXAMPLE VII

Bcl-G$_S$ is Associated with Cytosolic Organelles

Many Bcl-2 family proteins, such as Bcl-2,
5   Bcl-X$_L$, and Bak, contain a hydrophobic stretch of amino-
acids near their carboxyl-terminus that anchors them in
intracellular membranes of mitochondria, endoplasmic
reticulum, or nuclear envelope (reviewed in Reed, J. C.,
Nature, 387:773-776 (1997); Adams & Cory, Science,
10   281:1322-1326 (1998); Gross et al., Genes Dev., 13:1899-
1911 (1999)). However, some pro-apoptotic Bcl-2 family
proteins, such as Bax, Bid, and Bim, are found in the
cytosol and must be induced to translocate to
mitochondria and other organelles where the Bcl-2-family
15   proteins to which they dimerize reside (Wolter et al., J.
Cell Biol., 139:1281-1292 (1997); Puthalakath et al.,
Mol. Cell, 3:287-96 (1999); Li et al., Cell, 94:491-501
(1998); Luo et al., Cell, 94:481-490 (1998)).

The intracellular locations of the Bcl-G$_L$ and
20   Bcl-G$_S$ protein was examined by confocal microscopy
analysis of cells expressing GFP-tagged proteins.
GFP-expressing cells were imaged by confocal microscopy
using a Bio-Rad MRC 1024 instrument (Xu & Reed, supra.
(1998); Zhang et al., supra. (2000); Zha et al., Mol.
25   Cell. Biol., 16:6494-6508 (1996)). GFP-Bcl-G$_L$ protein was
located diffusely throughout cells, similar to GFP
control protein (Figure 11A, B). In contrast, Bcl-G$_S$ was
found in a punctate cytosolic pattern (Figure 11C),
suggestive of organelle association. Surprisingly,
30   deletion of the BH3 domain from Bcl-G$_S$ did not disrupt the
punctate distribution (Figure 5D), indicating that other
regions of the Bcl-G$_S$ protein are sufficient for

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subcellular targeting. Subcellular fractionation
experiments confirmed these observations, demonstrating
association of Bcl-G$_s$ and Bcl-G$_s$ (DB43) predominantly with
organelle-containing heavy-membrane fractions, with scant

5   amounts in the soluble cytosolic compartment.

EXAMPLE VIII
Loss of Heterozygosity (LOH) is Associated with Bcl-G in Ovarian Tissue

        This example describes loss of heterozygosity
10   (LOH) associated with Bcl-G in ovarian cancer tissue.

        Ovarian cancer tissue samples were tested for
SSCP for possible mutations in Bcl-G. No mutation was
found in exon 1. However, about one third of the ovarian
samples showed a possible LOH of Bcl-G. The LOH was
15   observed as a change in band intensity using SSCP. The
results were confirmed independently using PCR. The LOH
samples are sequenced to determine specific mutations.

        These results indicate that LOH is associated
with Bcl-G in ovarian tissue and can be useful as a
20   marker for ovarian cancer.

Example IX
Cloning of Mouse Bcl-G

        This example describes cloning of mouse Bcl-G.

        The mouse Bcl-G was identified by searching
25   GenBank. An EST clone (AA536718) was found to contain
mouse Bcl-G. The EST was purchased from the American
Type Culture Collection (ATCC; Manassas VA) and sequenced

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to determine the complete sequence of mouse Bcl-G.

The nucleotide sequence of mouse Bcl-G cDNA is referenced as SEQ ID NO:41. The amino acid sequence of Bcl-G is referenced as SEQ ID NO:42.

5   PCR was used to isolate mouse Bcl-G from the purchased EST clone and clone it into EGFP-C1 vector. he primers used were MXSTA, 5'-GGGCTCGAGATGTGCAGCACCAGTGTGTATG-3' (SEQ ID NO:43); NHREV, 5'-CCAAGCTTTAAGTCTACTTCTTCATGTGATATCCC-3' (SEQ ID

10   NO:44).

In preliminary experiments, mouse Bcl-G was overexpressed in Cos-7 and 293T cells. In these preliminary experiments, apoptosis was not observed.

Throughout this application various

15   publications have been referenced. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

20   Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the claims.

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What is claimed is:

    1.   An isolated nucleic acid encoding a Bcl-G
polypeptide, or a functional fragment thereof.

    2.   An isolated nucleic acid encoding a Bcl-G
 5   polypeptide, or a functional fragment thereof, comprising
a nucleic acid selected from:
        (a)   nucleic acid encoding the amino acid
sequence set forth in SEQ ID NOS:2, 4 or 42, or
        (b)   nucleic acid that hybridizes to the
10   nucleic acid of (a) under moderately stringent
conditions, wherein said nucleic acid contiguously
encodes biologically active Bcl-G, or
        (c)   nucleic acid degenerate with respect to
either (a) or (b) above, wherein said nucleic acid
15   encodes biologically active Bcl-G.

    3.   The nucleic acid of claim 2, wherein said
nucleic acid hybridizes under high stringency conditions
to the Bcl-G coding portion of any of SEQ ID NOS:1, 3 or
41.

20   4.   The nucleic acid of claim 2, wherein the
nucleotide sequence of said nucleic acid is substantially
the same as set forth in any of SEQ ID NOS:1, 3 or 41.

    5.   The nucleic acid of claim 2, wherein the
nucleotide sequence of said nucleic acid is the same as
25   that set forth in any of SEQ ID NOS:1, 3 or 41, or a
modification thereof.

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6. The nucleic acid of claim 2, wherein said nucleic acid is cDNA.

7. A vector containing the nucleic acid of claim 2.

8. Recombinant cells containing the nucleic acid of claim 2.

9. A Bcl-G oligonucleotide, comprising between 15 and 300 contiguous nucleotides of SEQ ID NOS:1, 3 or 41 or the anti-sense strand thereof.

10. An oligonucleotide according to claim 9, wherein said oligonucleotide is labeled with a detectable marker.

11. An antisense-nucleic acid capable of specifically binding to mRNA encoded by said nucleic acid according to claim 2.

12. A kit for detecting the presence of a Bcl-G nucleic acid sequence comprising at least one oligonucleotide according to claim 10.

13. An isolated Bcl-G polypeptide, or a functional fragment thereof, encoded by the nucleic acid of claim 2.

14. The Bcl-G polypeptide of claim 13, wherein said polypeptide comprises the substantially the same amino acid sequence of Bcl-G$_L$ (SEQ ID NO:2), Bcl-G$_S$ (SEQ ID NO:4), or mouse Bcl-G (SEQ ID NO:42).

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15.   The Bcl-G polypeptide of claim 14, wherein said polypeptide comprises the amino acid sequence of Bcl-G$_L$ (SEQ ID NO:2), Bcl-G$_S$ (SEQ ID NO:4), or mouse Bcl-G (SEQ ID NO:42).

5

16.   The Bcl-G polypeptide of claim 13, wherein said functional fragment comprises a BH3 or BH2 domain.

17.   The Bcl-G polypeptide of claim 16, wherein said functional fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 6, 9

10   and 18.   .

18.   The Bcl-G polypeptide of claim 13, wherein said polypeptide is encoded by a nucleotide sequence comprising substantially the same nucleotide sequence as set forth in SEQ ID NOS:1, 3 or 41.

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19.   The Bcl-G polypeptide of claim 13, wherein said polypeptide is encoded by a nucleotide sequence comprising the sequence set forth in SEQ ID NOS:1 or 3.

20.   A method for expression of a Bcl-G polypeptide, said method comprising culturing cells of

20   claim 8 under conditions suitable for expression of said Bcl-G.

21.   An isolated anti-Bcl-G antibody having specific reactivity with a Bcl-G according to claim 13.

22.   Antibody according to claim 21, wherein

25   said antibody is a monoclonal antibody.

23.   A cell line producing the monoclonal antibody of claim 22.

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24. An antibody according to claim 21, wherein said antibody is a polyclonal antibody.

25. A composition comprising an amount of the antisense-nucleic acid according to claim 11 effective to inhibit expression of a human Bcl-G and an acceptable carrier capable of delivering Bcl-G to a cell.

26. A transgenic nonhuman mammal expressing exogenous nucleic acid according to claim 2, encoding a Bcl-G.

27. A transgenic nonhuman mammal according to claim 26, wherein said nucleic acid encoding said Bcl-G has been mutated, and wherein the Bcl-G so expressed is not native Bcl-G.

28. A mutant non-human mammal having a disrupted Bcl-G gene.

29. A transgenic nonhuman mammal according to claim 26, wherein the transgenic nonhuman mammal is a mouse.

30. A method for identifying nucleic acids encoding a mammalian Bcl-G, comprising contacting a sample containing nucleic acids with one or more oligonucleotides according to claim 9, wherein said contacting is effected under high stringency hybridization conditions, and identifying a nucleic acid that hybridizes to said oligonucleotide.

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31. A method of detecting a Bcl-G nucleic acid molecule in a sample, comprising contacting said sample with two or more Bcl-G oligonucleotides of claim 9, amplifying a nucleic acid molecule, and detecting said amplification.

32. The method of claim 31, wherein said amplification is performed using polymerase chain reaction.

33. A method for detecting the presence of a human Bcl-G in a sample, comprising contacting a sample with an antibody according to claim 21, and detecting the presence of specific binding of said antibody to said sample, thereby detecting the presence of a human Bcl-G in said sample.

34. Single stranded nucleic acid primers for amplification of a Bcl-G nucleic acid, wherein said primers comprise a nucleic acid sequence derived from the nucleic acid sequences set forth as SEQ ID NOS:1 or 3.

35. A method for modulating the activity of an oncogenic polypeptide, comprising contacting said oncogenic polypeptide with a substantially pure Bcl-G, or an oncogenic protein-binding fragment thereof.

36. A method of identifying an effective agent that alters the association of a Bcl-G with a Bcl-G associated polypeptide (BAP), comprising the steps of:

(a) contacting said Bcl-G and said BAP polypeptide, under conditions that allow said Bcl-G and BAP polypeptide to associate, with a compound; and

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(b)   detecting the altered association of said
Bcl-G and BAP polypeptide, thereby identifying
a compound that is an effective agent for
altering the association of said Bcl-G with
5   BAP.

37.   The method of claim 36, wherein said
compound is a drug.

38.   The method of claim 36, wherein said
compound is a polypeptide.

10   39.   A method for modulating an activity
mediated by a Bcl-G polypeptide, comprising contacting
said Bcl-G polypeptide with an effective, modulating
amount of an agent identified by claim 36.

40.   The method of claim 39, wherein said
15   modulated activity is the binding of Bcl-G to a Bcl-2
family member.

41.   A method of modulating the level apoptosis
in a cell, comprising the steps of:

(a)   introducing a nucleic acid molecule
20   encoding a Bcl-G into the cell; and

(b)   expressing said Bcl-G in said cell,
wherein the expression of said Bcl-G modulates
apoptosis in said cell.

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42.   A method of modulating the level of apoptosis in a cell, comprising introducing an antisense nucleotide sequence into the cell, wherein said antisense nucleotide sequence specifically hybridizes to a nucleic acid molecule encoding a Bcl-G, wherein said hybridization reduces or inhibits the expression of said Bcl-G in said cell.

43.   A therapeutic composition comprising a pharmaceutically acceptable carrier and a compound selected from the group consisting of a Bcl-G polypeptide, a functional fragment of said Bcl-G, a Bcl-G modulating compound identified according to claim 36, and an anti-Bcl-G antibody.

44.   A method of treating a pathology characterized by abnormal cell proliferation, comprising administering an effective amount of the composition according to claim 43.

45.   A method of diagnosing a pathology characterized by an increased or decreased level of a Bcl-G in a subject, comprising the steps of:

(a)   obtaining a test sample from the subject;

(b)   contacting said sample with an agent that can bind said Bcl-G under suitable conditions, wherein said conditions allow specific binding of said agent to said Bcl-G; and

(c)   comparing the amount of said specific binding in said test sample with the amount of specific binding in a control sample, wherein an increased or decreased amount of said

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specific binding in said test sample as
compared to said control sample is diagnostic
of a pathology.

46.   The method of claim 45, wherein said agent
is selected from the group consisting of an anti-Bcl-G
antibody, a Bcl-G-associated-protein (BAP), and a Bcl-G
nucleic acid.

47.   A method of modulating the level of
apoptosis in a cell, comprising contacting the cell with
a compound that effectively alters the association of
Bcl-G with a Bcl-G-associated-protein in the cell, or
that effectively alters the activity of a Bcl-G in the
cell.

48.   A chimeric protein comprising a domain
selected from the group consisting of BH3 (SEQ ID NOS:5
or 9) and BH2 (SEQ ID NOS:6 or 18).

49.   A method of modulating interactions
between Bcl-G and Bcl-2, comprising contacting a Bcl-G
polypeptide with the agent of claim 36.

50.   A method of diagnosing cancer or
monitoring cancer therapy comprising contacting a test
sample from a patient with the antibody of claim 21.

51.   A method of assessing prognosis of
patients with cancer comprising contacting a test sample
from a patient with the antibody of claim 21.

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52. A method of diagnosing cancer or monitoring cancer therapy comprising contacting a test sample from a patient with the oligonucleotide of claim 9.

53. A method of assessing prognosis of patients with cancer comprising contacting a test sample from a patient with the oligonucleotide of claim 9.

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ccaagctggagaccacgaggattcagggactgaagccagc  80
atgggaattcctggtttgagatcagagtcctgagtacctc  120
gtgggaacttgggcactcatccgcaggaggtctagacccc  160
cagagaattccttgagtctaaggcacaggcccaacatgtg  200
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gatgatgacctaaacaccatagaattcaaaatcctcgcct  280
actacaccagacatcatgtcttcaagagcacccctgctct  320
cttctcaccaaagctgctgagaacaagaagtttgtcccag  360
aggggcctggggaattgttcagcaaatgagtcatggacag  400
             410         420         430         440
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ccaagattcgcacagccagcagtggtccaggtgtctttct  600
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agctccaaggccacgtgcctgtagcttcaagttctaagaa  800
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ggtgtggaccccaggggagaatcagaggtcaaagctcagg  1000
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tccagcagcacggtggatgggaaaaaatacttgggatatc  1160
acatgaagaagtagactga 1179
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FIGURE 1

Bcl-G_L

```
  1 - ATGTGTAGCACCAGTGGGTGTGACCTGGAAGAAATCCCCCTAGATGATGATGACCTAAAC - 60
    -  M  C  S  T  S  G  C  D  L  E  E  I  P  L  D  D  D  D  L  N
 61 - ACCATAGAATTCAAAATCCTCGCCTACTACACCAGACATCATGTCTTCAAGAGCACCCCT - 120
    -  T  I  E  F  K  I  L  A  Y  Y  T  R  H  H  V  F  K  S  T  P
121 - GCTCTCTTCTCACCAAAGCTGCTGAGAACAAGAAGTTTGTCCCAGAGGGGCCTGGGGAAT - 180
    -  A  L  F  S  P  K  L  L  R  T  R  S  L  S  Q  R  G  L  G  N
181 - TGTTCAGCAAATGAGTCATGGACAGAGGTGTCATGGCCTTGCAGAAATTCCCAATCCAGT - 240
    -  C  S  A  N  E  S  W  T  E  V  S  W  P  C  R  N  S  Q  S  S
241 - GAGAAGGCCATAAACCTTGGCAAGAAAAAGTCTTCTTGGAAAGCATTCTTTGGAGTAGTG - 300
    -  E  K  A  I  N  L  G  K  K  K  S  S  W  K  A  F  F  G  V  V
301 - GAGAAGGAAGATTCGCAGAGCACGCCTGCCAAGGTCTCTGCTCAGGGTCAAAGGACGTTG - 360
    -  E  K  E  D  S  Q  S  T  P  A  K  V  S  A  Q  G  Q  R  T  L
361 - GAATACCAAGATTCGCACAGCCAGCAGTGGTCCAGGTGTCTTTCTAACGTGGAGCAGTGC - 420
    -  E  Y  Q  D  S  H  S  Q  Q  W  S  R  C  L  S  N  V  E  Q  C
421 - TTGGAGCATGAAGCTGTGGACCCCAAAGTCATTTCCATTGCCAACCGAGTAGCTGAAATT - 480
    -  L  E  H  E  A  V  D  P  K  V  I  S  I  A  N  R  V  A  E  I
481 - GTTTACTCCTGGCCACCACCACAAGCGACCCAGGCAGGAGGCTTCAAGTCCAAAGAGATT - 540
    -  V  Y  S  W  P  P  P  Q  A  T  Q  A  G  G  F  K  S  K  E  I
541 - TTTGTAACTGAGGGTCTCTCCTTCCAGCTCCAAGGCCACGTGCCTGTAGCTTCAAGTTCT - 600
    -  F  V  T  E  G  L  S  F  Q  L  Q  G  H  V  P  V  A  S  S  S
601 - AAGAAAGATGAAGAAGAACAAATACTAGCCAAAATTGTTGAGCTGCTGAAATATTCAGGA - 660
    -  K  K  D  E  E  E  Q  I  L  A  K  I  V  E  L  L  K  Y  S  G
661 - GATCAGTTGGAAAGAAAGCTGAAGAAAGATAAGGCTTTGATGGGCCACTTCCAGGATGGG - 720
    -  D  Q  L  E  R  K  L  K  K  D  K  A  L  M  G  H  F  Q  D  G
721 - CTGTCCTACTCTGTTTTCAAGACCATCACAGACCAGGTCCTAATGGGTGTGGACCCCAGG - 780
    -  L  S  Y  S  V  F  K  T  I  T  D  Q  V  L  M  G  V  D  P  R
781 - GGAGAATCAGAGGTCAAAGCTCAGGGCTTTAAGGCTGCCCTTGTAATAGACGTCACGGCC - 840
    -  G  E  S  E  V  K  A  Q  G  F  K  A  A  L  V  I  D  V  T  A
841 - AAGCTCACAGCTATTGACAACCACCCGATGAACAGGGTCCTGGGCTTTGGCACCAAGTAC - 900
    -  K  L  T  A  I  D  N  H  P  M  N  R  V  L  G  F  G  T  K  Y
901 - CTGAAAGAGAACTTCTCGCCATGGATCCAGCAGCACGGTGGATGGGAAAAAATACTTGGG - 960
    -  L  K  E  N  F  S  P  W  I  Q  Q  H  G  G  W  E  K  I  L  G
961 - ATATCACATGAAGAAGTAGACTGA - 984
    -  I  S  H  E  E  V  D  *
```

FIGURE 2

```
            10        20        30        40
    ┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴
    aatgacatgacagccattccgtggccagggacaccactgc  40
    ccaagctggagaccacgaggattcagggactgaagccagc  80
    atgggaattcctggtttgagatcagagtcctgagtacctc  120
    gtgggaacttgggcactcatccgcaggaggtctagacccc  160
    cagagaattccttgagtctaaggcacaggcccaacatgtg  200

           210       220       230       240
    ┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴
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    actacaccagacatcatgtcttcaagagcacccctgctct  320
    cttctcaccaaagctgctgagaacaagaagtttgtcccag  360
    aggggcctggggaattgttcagcaaatgagtcatggacag  400

           410       420       430       440
    ┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴
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    ctgccaaggtctctgctcagggtcaaaggacgttggaata  560
    ccaagattcgcacagccagcagtggtccaggtgtctttct  600

           610       620       630       640
    ┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴
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    aagtcatttccattgccaaccgagtagctgaaattgttta  680
    ctcctggccaccaccacaagcgacccaggcaggaggcttc  720
    aagtccaaagagattttttgtaactgagggtctctccttcc  760
    agctccaaggccacgtgcctgtagcttcaagttctaagaa  800

           810       820       830       840
    ┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴
    agatgaagaagaacaaatactagccaaaattgttgagctg  840
    ctgaaatattcaggagatcagttggaaagaaaggacactg  880
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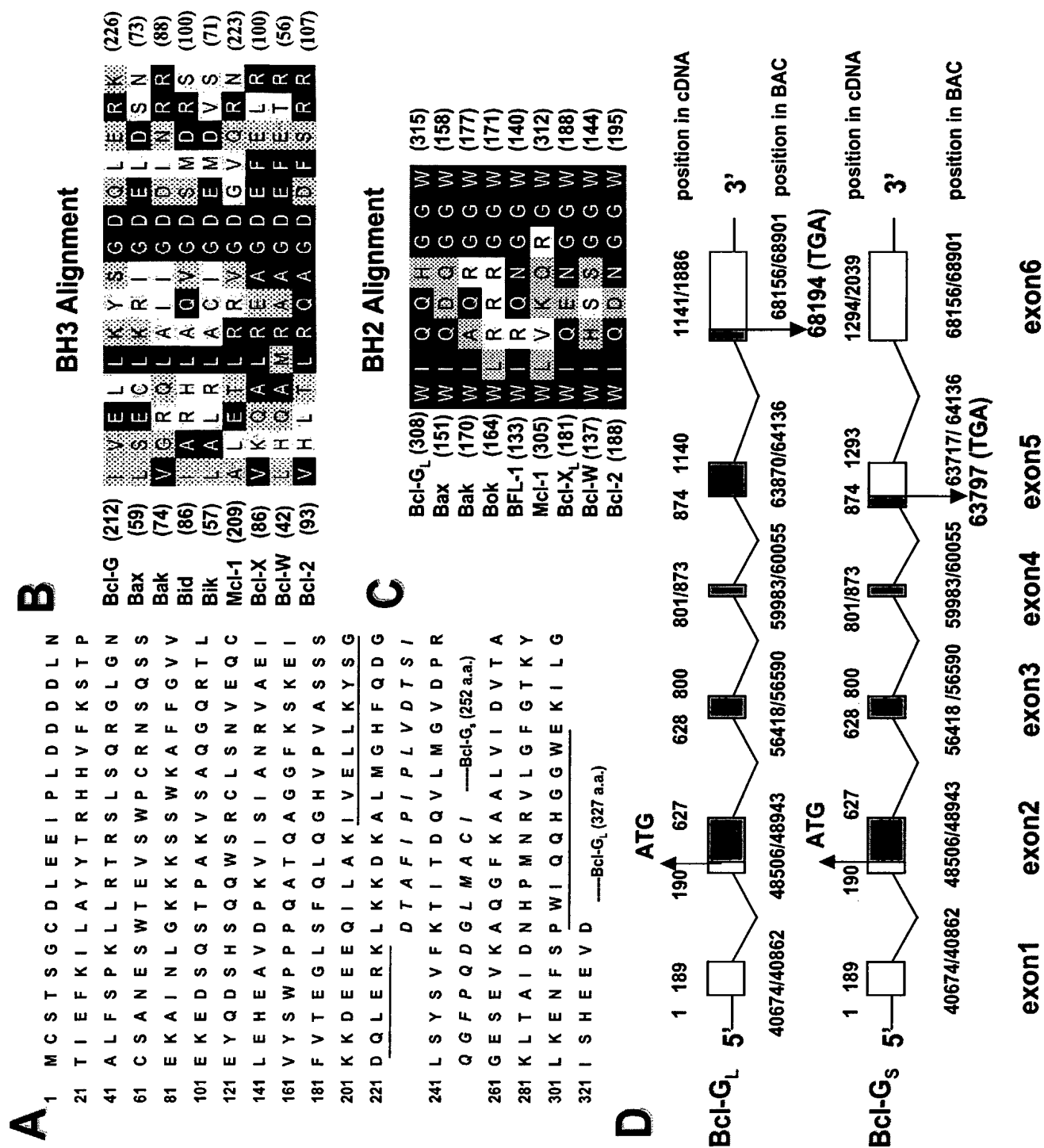
FIGURE 3

3/11

Bcl-G_S

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  1 - ATGTGTAGCACCAGTGGGTGTGACCTGGAAGAAATCCCCCTAGATGATGATGACCTAAAC -  60
    - M   C   S   T   S   G   C   D   L   E   E   I   P   L   D   D   D   L   N
 61 - ACCATAGAATTCAAAAATCCTCGCCTACTACACCAGACACATCATGTCTTCAAGAGCACCCT - 120
    - T   I   E   F   K   I   L   A   Y   Y   T   R   H   H   V   F   K   S   T   P
121 - GCTCTCTTCTCACCAAAGCTGCTGAGAACAAGAAGTTTGTCCCAGAGGGCCTGGGGAAT - 180
    - A   L   F   S   P   K   L   L   R   T   R   S   L   S   Q   R   G   L   G   N
181 - TGTTCAGCAAATGAGTCATGGACAGAGGTGTCATGGCCTTGCAGAAATTCCCAATCCAGT - 240
    - C   S   A   N   E   S   W   T   E   V   S   W   P   C   R   N   S   Q   S   S
241 - GAGAAGGCCATAAACCTTGGCAAGAAAAAGTCTTCTTGGAAAGCATTCTTTGGAGTAGTG - 300
    - E   K   A   I   N   L   G   K   K   K   S   S   W   K   A   F   F   G   V   V
301 - GAGAAGGAAGATTCGCAGAGCACGCCTGCCAAGGTCTCTGCTCAGGGTCAAAGGACGTTG - 360
    - E   K   E   D   S   Q   S   T   P   A   K   V   S   A   Q   G   Q   R   T   L
361 - GAATACCAAGATTCGCACAGCCAGCAGTGGTCCAGGTGTCTTCTAACGTGGAGCAGTGC - 420
    - E   Y   Q   D   S   H   S   Q   Q   W   S   R   C   L   S   N   V   E   Q   C
421 - TTGGAGCATGAAGCTGTGGACCCCAAAGTCATTCCATTGCCAACCGAGTAGCTGAAATT - 480
    - L   E   H   E   A   V   D   P   K   V   I   S   I   A   N   R   V   A   E   I
481 - GTTTACTCCTGGCCACCACCAAGCAGGCCAGGCAGGAGGCTTCAAGTCCAAAGAGATT - 540
    - V   Y   S   W   P   P   P   Q   A   T   Q   A   G   G   F   K   S   K   E   I
541 - TTTGTAACTGAGGGTCTCTCCTTCCAGCTCCAAGGCCATGCCTGTAGCTTCAAGTTCT - 600
    - F   V   T   E   G   L   S   F   Q   L   Q   G   H   V   P   V   A   S   S   S
601 - AAGAAAGATGAAGAAGAAGAACAAAATTGTTGAGCTGCTGAAATATTCAGGA - 660
    - K   K   D   E   E   Q   I   L   A   K   I   V   E   L   L   K   Y   S   G
661 - GATCAGTTGGAAAGAAGGACACTGCCTTCATCCCATTCCCTGGTTGACACCAGCATC - 720
    - D   Q   L   E   R   K   D   T   A   F   I   P   I   P   L   V   D   T   S   I
721 - CAGGGGTTTTCCACAGGATGGTTTGATGGGCTGATGGCCTGCATTGA - 759
    - Q   G   F   P   Q   D   G   L   M   A   C   I   *
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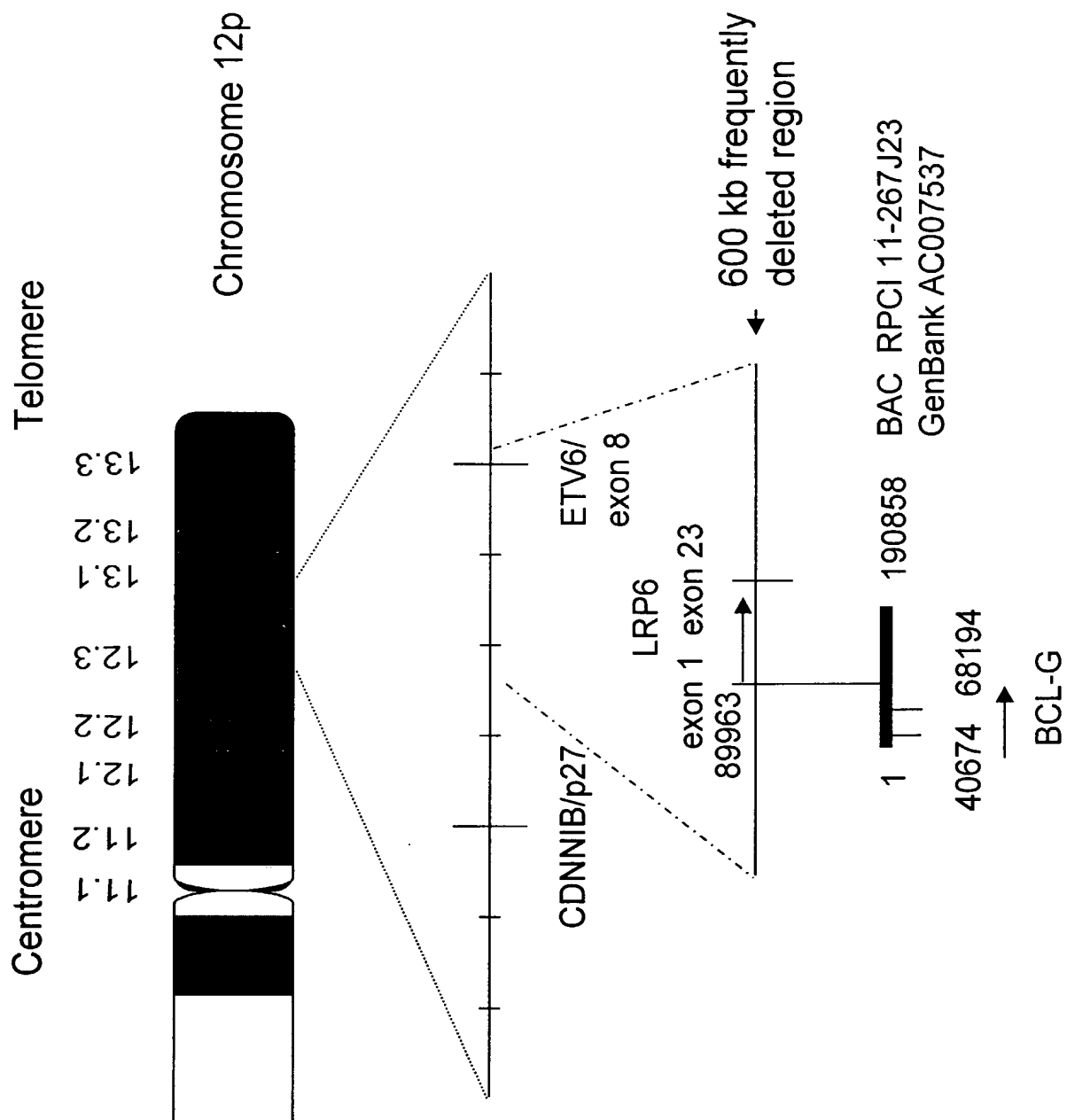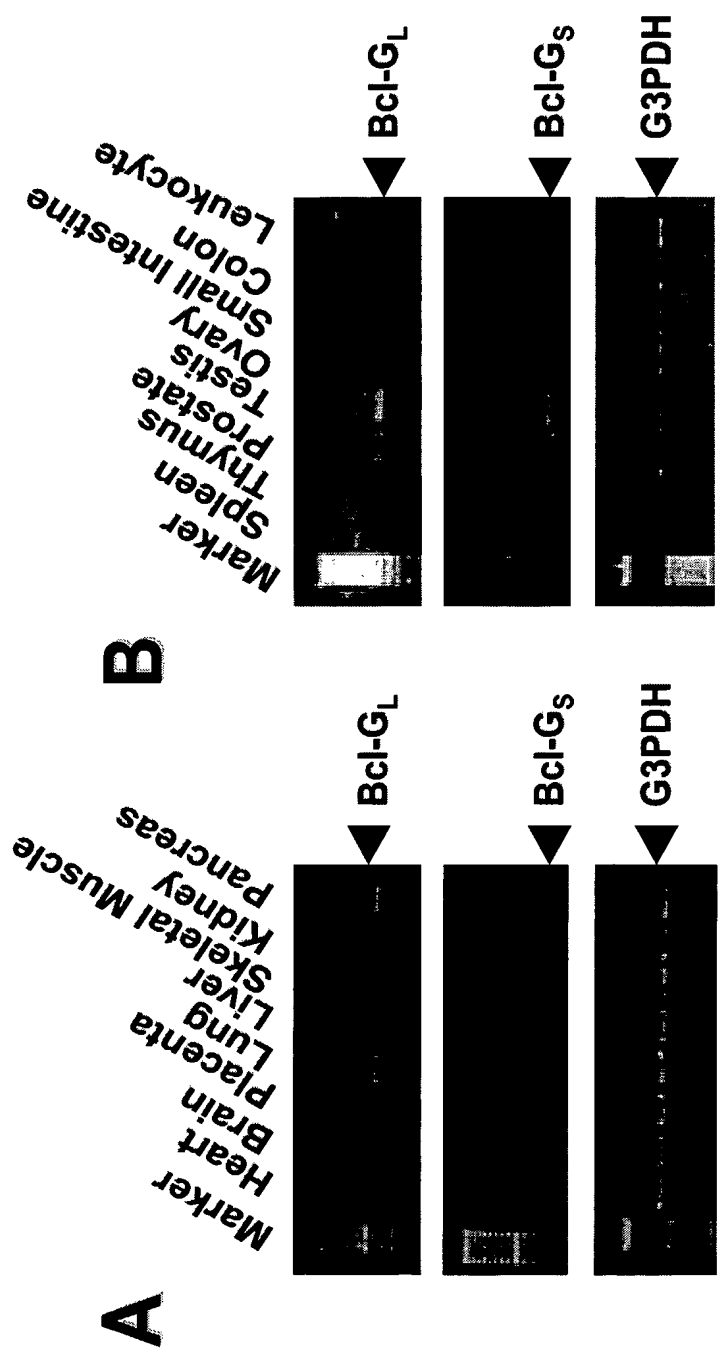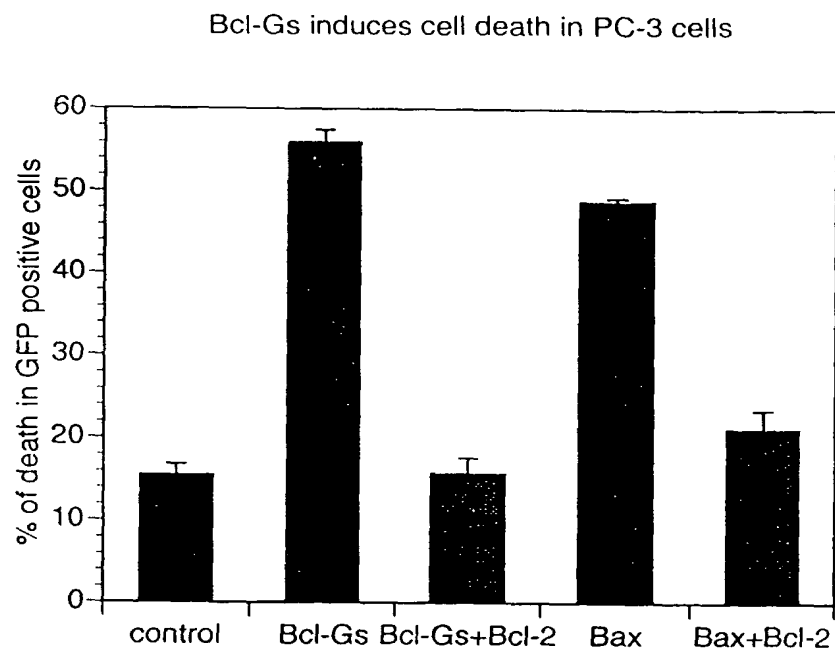
FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 7

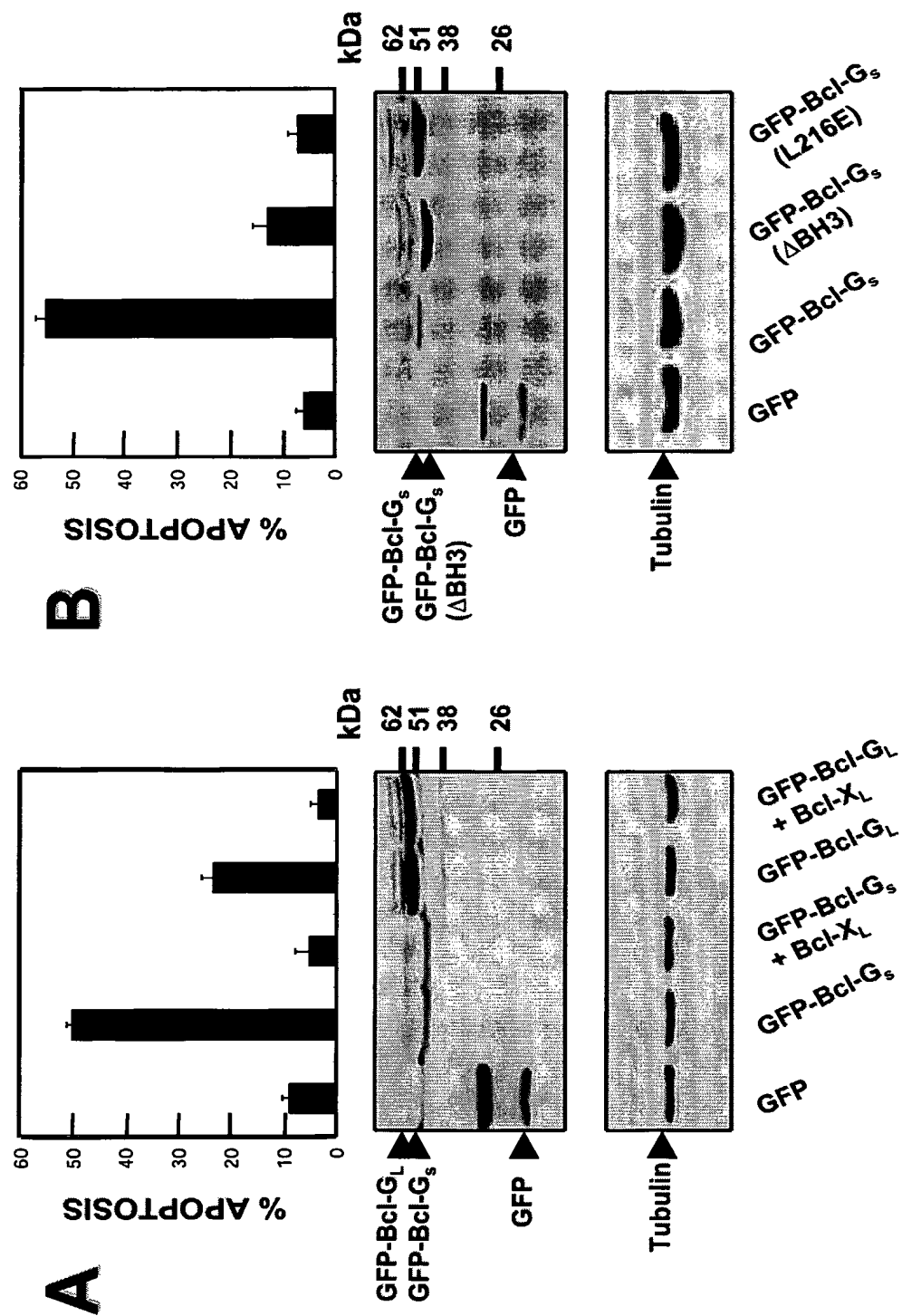Bcl-Gs induces cell death in PC-3 cells
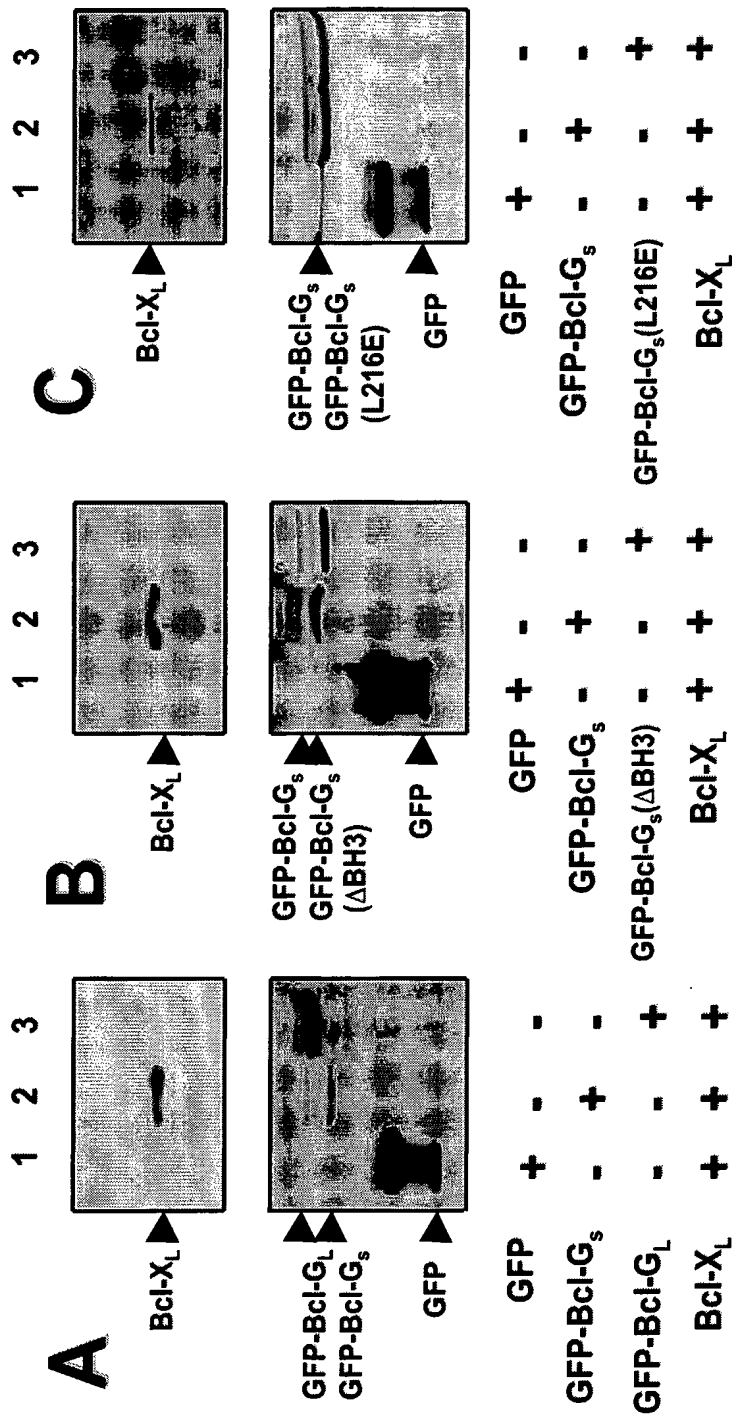
FIGURE 8

FIGURE 9

FIGURE 10
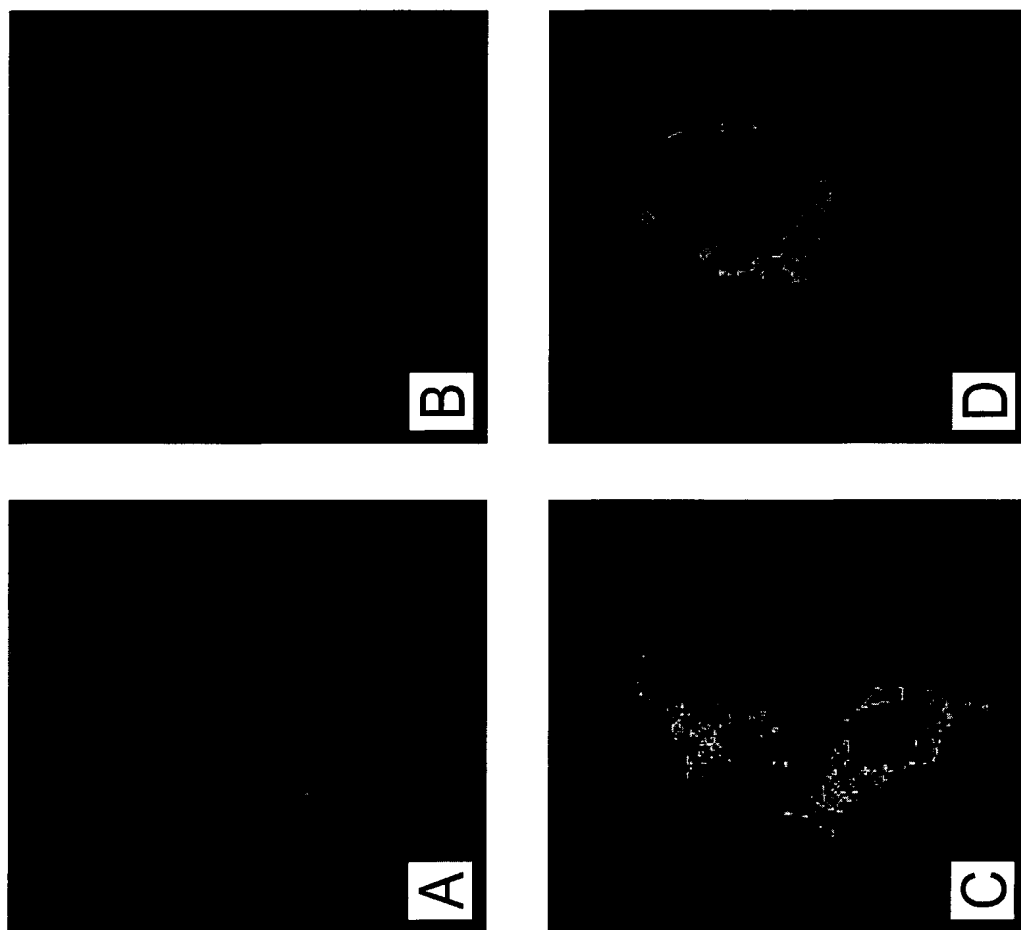
FIGURE 11

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      Godzik, Adam
      The Burnham Institute

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gtgggaactt gggcactcat ccgcaggagg tctagacccc cagagaattc cttgagtcta 180

aggcacaggc ccaac atg tgt agc acc agt ggg tgt gac ctg gaa gaa atc   231
                  Met Cys Ser Thr Ser Gly Cys Asp Leu Glu Glu Ile
                   1   5   10

ccc cta gat gat gat gac cta aac acc ata gaa ttc aaa atc ctc gcc   279
Pro Leu Asp Asp Asp Asp Leu Asn Thr Ile Glu Phe Lys Ile Leu Ala
         15   20   25

tac tac acc aga cat cat gtc ttc aag agc acc cct gct ctc ttc tca   327
Tyr Tyr Thr Arg His His Val Phe Lys Ser Thr Pro Ala Leu Phe Ser
     30   35   40

cca aag ctg ctg aga aca aga agt ttg tcc cag agg ggc ctg ggg aat   375
Pro Lys Leu Leu Arg Thr Arg Ser Leu Ser Gln Arg Gly Leu Gly Asn
 45   50   55   60

tgt tca gca aat gag tca tgg aca gag gtg tca tgg cct tgc aga aat   423
Cys Ser Ala Asn Glu Ser Trp Thr Glu Val Ser Trp Pro Cys Arg Asn
                 65   70   75

1

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tcc caa tcc agt gag aag gcc ata aac ctt ggc aag aaa aag tct tct    471
Ser Gln Ser Ser Glu Lys Ala Ile Asn Leu Gly Lys Lys Lys Ser Ser
            80                  85                  90

tgg aaa gca ttc ttt gga gta gtg gag aag gaa gat tcg cag agc acg    519
Trp Lys Ala Phe Phe Gly Val Val Glu Lys Glu Asp Ser Gln Ser Thr
            95                 100                 105

cct gcc aag gtc tct gct cag ggt caa agg acg ttg gaa tac caa gat    567
Pro Ala Lys Val Ser Ala Gln Gly Gln Arg Thr Leu Glu Tyr Gln Asp
        110                 115                 120

tcg cac agc cag cag tgg tcc agg tgt ctt tct aac gtg gag cag tgc    615
Ser His Ser Gln Gln Trp Ser Arg Cys Leu Ser Asn Val Glu Gln Cys
125                 130                 135                 140

ttg gag cat gaa gct gtg gac ccc aaa gtc att tcc att gcc aac cga    663
Leu Glu His Glu Ala Val Asp Pro Lys Val Ile Ser Ile Ala Asn Arg
                145                 150                 155

gta gct gaa att gtt tac tcc tgg cca cca cca caa gcg acc cag gca    711
Val Ala Glu Ile Val Tyr Ser Trp Pro Pro Pro Gln Ala Thr Gln Ala
                160                 165                 170

gga ggc ttc aag tcc aaa gag att ttt gta act gag ggt ctc tcc ttc    759
Gly Gly Phe Lys Ser Lys Glu Ile Phe Val Thr Glu Gly Leu Ser Phe
            175                 180                 185

cag ctc caa ggc cac gtg cct gta gct tca agt tct aag aaa gat gaa    807
Gln Leu Gln Gly His Val Pro Val Ala Ser Ser Ser Lys Lys Asp Glu
        190                 195                 200

gaa gaa caa ata cta gcc aaa att gtt gag ctg ctg aaa tat tca gga    855
Glu Glu Gln Ile Leu Ala Lys Ile Val Glu Leu Leu Lys Tyr Ser Gly
205                 210                 215                 220

gat cag ttg gaa aga aag ctg aag aaa gat aag gct ttg atg ggc cac    903
Asp Gln Leu Glu Arg Lys Leu Lys Lys Asp Lys Ala Leu Met Gly His
                225                 230                 235

ttc cag gat ggg ctg tcc tac tct gtt ttc aag acc atc aca gac cag    951
Phe Gln Asp Gly Leu Ser Tyr Ser Val Phe Lys Thr Ile Thr Asp Gln
            240                 245                 250

gtc cta atg ggt gtg gac ccc agg gga gaa tca gag gtc aaa gct cag    999
Val Leu Met Gly Val Asp Pro Arg Gly Glu Ser Glu Val Lys Ala Gln
            255                 260                 265

ggc ttt aag gct gcc ctt gta ata gac gtc acg gcc aag ctc aca gct    1047
Gly Phe Lys Ala Ala Leu Val Ile Asp Val Thr Ala Lys Leu Thr Ala
        270                 275                 280

att gac aac cac ccg atg aac agg gtc ctg ggc ttt ggc acc aag tac    1095
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285                 290                 295                 300

ctg aaa gag aac ttc tcg cca tgg atc cag cag cac ggt gga tgg gaa    1143
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2

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His His Val Phe Lys Ser Thr Pro Ala Leu Phe Ser Pro Lys Leu Leu
            35                  40                  45

Arg Thr Arg Ser Leu Ser Gln Arg Gly Leu Gly Asn Cys Ser Ala Asn
        50                  55                  60

Glu Ser Trp Thr Glu Val Ser Trp Pro Cys Arg Asn Ser Gln Ser Ser
 65                  70                  75                  80

Glu Lys Ala Ile Asn Leu Gly Lys Lys Lys Ser Ser Trp Lys Ala Phe
                85                  90                  95

Phe Gly Val Val Glu Lys Glu Asp Ser Gln Ser Thr Pro Ala Lys Val
            100                 105                 110

Ser Ala Gln Gly Gln Arg Thr Leu Glu Tyr Gln Asp Ser His Ser Gln
        115                 120                 125

Gln Trp Ser Arg Cys Leu Ser Asn Val Glu Gln Cys Leu Glu His Glu
    130                 135                 140

Ala Val Asp Pro Lys Val Ile Ser Ile Ala Asn Arg Val Ala Glu Ile
145                 150                 155                 160

Val Tyr Ser Trp Pro Pro Pro Gln Ala Thr Gln Ala Gly Gly Phe Lys
                165                 170                 175

Ser Lys Glu Ile Phe Val Thr Glu Gly Leu Ser Phe Gln Leu Gln Gly
                180                 185                 190

His Val Pro Val Ala Ser Ser Ser Lys Lys Asp Glu Glu Glu Gln Ile
            195                 200                 205

Leu Ala Lys Ile Val Glu Leu Leu Lys Tyr Ser Gly Asp Gln Leu Glu
        210                 215                 220

Arg Lys Leu Lys Lys Asp Lys Ala Leu Met Gly His Phe Gln Asp Gly
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225                      230                      235                      240

Leu Ser Tyr Ser Val Phe Lys Thr Ile Thr Asp Gln Val Leu Met Gly
            245                  250                  255

Val Asp Pro Arg Gly Glu Ser Glu Val Lys Ala Gln Gly Phe Lys Ala
            260                  265                  270

Ala Leu Val Ile Asp Val Thr Ala Lys Leu Thr Ala Ile Asp Asn His
            275                  280                  285

Pro Met Asn Arg Val Leu Gly Phe Gly Thr Lys Tyr Leu Lys Glu Asn
        290                  295                  300

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gtgggaactt gggcactcat ccgcaggagg tctagacccc cagagaattc cttgagtcta  180

aggcacaggc ccaac atg tgt agc acc agt ggg tgt gac ctg gaa gaa atc  231
                  Met Cys Ser Thr Ser Gly Cys Asp Leu Glu Glu Ile
                   1               5                  10

ccc cta gat gat gat gac cta aac acc ata gaa ttc aaa atc ctc gcc  279
Pro Leu Asp Asp Asp Asp Leu Asn Thr Ile Glu Phe Lys Ile Leu Ala
        15                  20                  25

tac tac acc aga cat cat gtc ttc aag agc acc cct gct ctc ttc tca  327
Tyr Tyr Thr Arg His His Val Phe Lys Ser Thr Pro Ala Leu Phe Ser
    30                  35                  40

cca aag ctg ctg aga aca aga agt ttg tcc cag agg ggc ctg ggg aat  375
Pro Lys Leu Leu Arg Thr Arg Ser Leu Ser Gln Arg Gly Leu Gly Asn
45                  50                  55                  60

tgt tca gca aat gag tca tgg aca gag gtg tca tgg cct tgc aga aat  423
Cys Ser Ala Asn Glu Ser Trp Thr Glu Val Ser Trp Pro Cys Arg Asn
                65                  70                  75
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```
tcc caa tcc agt gag aag gcc ata aac ctt ggc aag aaa aag tct tct     471
Ser Gln Ser Ser Glu Lys Ala Ile Asn Leu Gly Lys Lys Lys Ser Ser
             80              85              90

tgg aaa gca ttc ttt gga gta gtg gag aag gaa gat tcg cag agc acg     519
Trp Lys Ala Phe Phe Gly Val Val Glu Lys Glu Asp Ser Gln Ser Thr
         95              100             105

cct gcc aag gtc tct gct cag ggt caa agg acg ttg gaa tac caa gat     567
Pro Ala Lys Val Ser Ala Gln Gly Gln Arg Thr Leu Glu Tyr Gln Asp
     110             115             120

tcg cac agc cag cag tgg tcc agg tgt ctt tct aac gtg gag cag tgc     615
Ser His Ser Gln Gln Trp Ser Arg Cys Leu Ser Asn Val Glu Gln Cys
125             130             135             140

ttg gag cat gaa gct gtg gac ccc aaa gtc att tcc att gcc aac cga     663
Leu Glu His Glu Ala Val Asp Pro Lys Val Ile Ser Ile Ala Asn Arg
             145             150             155

gta gct gaa att gtt tac tcc tgg cca cca cca caa gcg acc cag gca     711
Val Ala Glu Ile Val Tyr Ser Trp Pro Pro Pro Gln Ala Thr Gln Ala
             160             165             170

gga ggc ttc aag tcc aaa gag att ttt gta act gag ggt ctc tcc ttc     759
Gly Gly Phe Lys Ser Lys Glu Ile Phe Val Thr Glu Gly Leu Ser Phe
         175             180             185

cag ctc caa ggc cac gtg cct gta gct tca agt tct aag aaa gat gaa     807
Gln Leu Gln Gly His Val Pro Val Ala Ser Ser Ser Lys Lys Asp Glu
     190             195             200

gaa gaa caa ata cta gcc aaa att gtt gag ctg ctg aaa tat tca gga     855
Glu Glu Gln Ile Leu Ala Lys Ile Val Glu Leu Leu Lys Tyr Ser Gly
205             210             215             220

gat cag ttg gaa aga aag gac act gcc ttc atc ccc att ccc ttg gtt     903
Asp Gln Leu Glu Arg Lys Asp Thr Ala Phe Ile Pro Ile Pro Leu Val
             225             230             235

gac acc agc atc cag ggt ttt cca cag gat ggt ttg atg gcc tgc att     951
Asp Thr Ser Ile Gln Gly Phe Pro Gln Asp Gly Leu Met Ala Cys Ile
             240             245             250

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His His Val Phe Lys Ser Thr Pro Ala Leu Phe Ser Pro Lys Leu Leu
            35   40   45

Arg Thr Arg Ser Leu Ser Gln Arg Gly Leu Gly Asn Cys Ser Ala Asn
        50   55   60

Glu Ser Trp Thr Glu Val Ser Trp Pro Cys Arg Asn Ser Gln Ser Ser
65   70   75   80

Glu Lys Ala Ile Asn Leu Gly Lys Lys Lys Ser Ser Trp Lys Ala Phe
                85   90   95

Phe Gly Val Val Glu Lys Glu Asp Ser Gln Ser Thr Pro Ala Lys Val
            100   105   110

Ser Ala Gln Gly Gln Arg Thr Leu Glu Tyr Gln Asp Ser His Ser Gln
        115   120   125

Gln Trp Ser Arg Cys Leu Ser Asn Val Glu Gln Cys Leu Glu His Glu
    130   135   140

Ala Val Asp Pro Lys Val Ile Ser Ile Ala Asn Arg Val Ala Glu Ile
145   150   155   160

Val Tyr Ser Trp Pro Pro Pro Gln Ala Thr Gln Ala Gly Gly Phe Lys
            165   170   175

Ser Lys Glu Ile Phe Val Thr Glu Gly Leu Ser Phe Gln Leu Gln Gly
            180   185   190

His Val Pro Val Ala Ser Ser Ser Lys Lys Asp Glu Glu Glu Gln Ile
        195   200   205

Leu Ala Lys Ile Val Glu Leu Leu Lys Tyr Ser Gly Asp Gln Leu Glu
    210   215   220

Arg Lys Asp Thr Ala Phe Ile Pro Ile Pro Leu Val Asp Thr Ser Ile
225   230   235   240

Gln Gly Phe Pro Gln Asp Gly Leu Met Ala Cys Ile
            245   250

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<210> 6

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<210> 16
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<400> 22
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 1               5
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  1               5


<210> 24
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<212> PRT
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<400> 24
Trp Ile Gln Glu Asn Gly Gly Trp
  1               5


<210> 25
<211> 8
<212> PRT
<213> Homo sapiens

<400> 25
Trp Ile His Ser Ser Gly Gly Trp
  1               5


<210> 26
<211> 8
<212> PRT
<213> Homo sapiens

<400> 26
Trp Ile Gln Asp Asn Gly Gly Trp
  1               5


<210> 27
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 27
ggctcgagcg atgtgtagca ccagtgggtg tgacc                    35


<210> 28
<211> 35
```

```
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 28
ccaagcttta agtctacttc ttcatgtgat atccc                     35


<210> 29
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 29
ccaagcttta aaatgcaggc catcaaacc                            29


<210> 30
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 30
ggctcgagcg atgtgtagca ccagtgggtg tgacc                     35


<210> 31
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 31
ccggatccgg ctagtatttg ttcttcttca tcttt                     35


<210> 32
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
```

primer

```
<400> 32
ccggatccga cactgccttc atccccattc cc                          32
```

```
<210> 33
<211> 29
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of Artificial Sequence:synthetic
      primer
```

```
<400> 33
ccaagcttta aaatgcaggc catcaaacc                              29
```

```
<210> 34
<211> 43
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of Artificial Sequence: synthetic
      primer
```

```
<400> 34
gccaaaattg ttgagctgga gaaatattca ggagatcagt tgg              43
```

```
<210> 35
<211> 43
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of Artificial Sequence: synthetic
      primer
```

```
<400> 35
ccaactgatc tcctgaatat ttctccagct caacaatttt ggc              43
```

```
<210> 36
<211> 28
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of Artificial Sequence: synthetic
      primer
```

```
<400> 36
ctgagggtct ctccttccag ctccaagg                               28
```

<210> 37
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 37
ggccgtgacg tctattacaa gggcagcc   28

<210> 38
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 38
caagggaatg gggatgaagg cagtgtc   27

<210> 39
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 39
tgaaggtcgg agtcaacgga tttggt   26

<210> 40
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 40
catgtgggcc atgaggtcca ccac   24

<210> 41
<211> 987
<212> DNA
<213> Mus musculus

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<220>
<221> CDS
<222> (1)..(987)

<220>
<221> unsure
<222> (319)
<223> unknown nucleotide

<400> 41
atg tgc agc acc agt gtg tat gac ctg gaa gac att ccc ctg gag gat      48
Met Cys Ser Thr Ser Val Tyr Asp Leu Glu Asp Ile Pro Leu Glu Asp
 1               5                  10                  15

gat gat cca aac agc ata gag ttc aaa atc ctg gcc ttc tac gcc aga      96
Asp Asp Pro Asn Ser Ile Glu Phe Lys Ile Leu Ala Phe Tyr Ala Arg
             20                  25                  30

cac cat gtc ttc aag aac acc ccg gct gtc ttc tcg ccc aag ctc tcc      144
His His Val Phe Lys Asn Thr Pro Ala Val Phe Ser Pro Lys Leu Ser
         35                  40                  45

aga aca agg agt ctg tcc cag aaa gcc ctg ggg act tgg tca act gat      192
Arg Thr Arg Ser Leu Ser Gln Lys Ala Leu Gly Thr Trp Ser Thr Asp
     50                  55                  60

tcc tgg aca cag gta tca ttg cct tgc aga ggt tcc ccc tcc agc gaa      240
Ser Trp Thr Gln Val Ser Leu Pro Cys Arg Gly Ser Pro Ser Ser Glu
 65                  70                  75                  80

aag aac atc agc ttg ggc aag aag aag tct tct tgg aga aca ctc ttc      288
Lys Asn Ile Ser Leu Gly Lys Lys Lys Ser Ser Trp Arg Thr Leu Phe
                 85                  90                  95

agg gtg gcc gag aag gag gaa ggc ctg ccg ngc tcc cca aag gag atc      336
Arg Val Ala Glu Lys Glu Glu Gly Leu Pro Xaa Ser Pro Lys Glu Ile
                 100                 105                 110

cga gct cag ggt cct cag ggc ccc ttc ccg gta gag cgg cag agt ggc      384
Arg Ala Gln Gly Pro Gln Gly Pro Phe Pro Val Glu Arg Gln Ser Gly
             115                 120                 125

ttc cac aac cag cac tgg ccc agg tct ctg agc agt gtg gag cag ccc      432
Phe His Asn Gln His Trp Pro Arg Ser Leu Ser Ser Val Glu Gln Pro
     130                 135                 140

tgg aga gtg aag ttg tgg att cca aag tgg ctt gta ttg cca aca gag      480
Trp Arg Val Lys Leu Trp Ile Pro Lys Trp Leu Val Leu Pro Thr Glu
145                 150                 155                 160

tgg ctg aaa ttg ttt act cct ggc cac cac cag atg tca tcc aca gcc      528
Trp Leu Lys Leu Phe Thr Pro Gly His His Gln Met Ser Ser Thr Ala
             165                 170                 175

agg gag gaa gcc agc tca aag aga ggg tct cgg aga ttt ttg tac ttc      576
Arg Glu Glu Ala Ser Ser Lys Arg Gly Ser Arg Arg Phe Leu Tyr Phe
```

14

```
          180                    185                    190

agg ttt gaa gga cct tgg gac tct aag aat aaa gat ggt gaa gac caa    624
Arg Phe Glu Gly Pro Trp Asp Ser Lys Asn Lys Asp Gly Glu Asp Gln
        195                    200                    205

ata ata agc aag att gtt gag ctg ctg aaa tcc tcg ggg gat cag ttg    672
Ile Ile Ser Lys Ile Val Glu Leu Leu Lys Ser Ser Gly Asp Gln Leu
        210                    215                    220

gga aga gag ata aag aaa gac aag gct ttg atg agc agc ttc cag gac    720
Gly Arg Glu Ile Lys Lys Asp Lys Ala Leu Met Ser Ser Phe Gln Asp
225                    230                    235                    240

ggg ctg tcc tac tca acg ttc aag acc atc aca gac ctg ttc ctg agg    768
Gly Leu Ser Tyr Ser Thr Phe Lys Thr Ile Thr Asp Leu Phe Leu Arg
                245                    250                    255

gac gtg gac acc aga gga gaa tca gag gtc aaa gct cgg ggc ttc aag    816
Asp Val Asp Thr Arg Gly Glu Ser Glu Val Lys Ala Arg Gly Phe Lys
            260                    265                    270

gct gcc ctt gca ata gac gcc atc gcc aag ctc acg gca tcg gac aac    864
Ala Ala Leu Ala Ile Asp Ala Ile Ala Lys Leu Thr Ala Ser Asp Asn
        275                    280                    285

cac cca atg aat aga atg ctg ggc ttc ggg acc aag tac cta aaa gag    912
His Pro Met Asn Arg Met Leu Gly Phe Gly Thr Lys Tyr Leu Lys Glu
    290                    295                    300

tac ttc tcc ccc tgg gtt cag cag aat ggc gga tgg gaa aaa ata ctt    960
Tyr Phe Ser Pro Trp Val Gln Gln Asn Gly Gly Trp Glu Lys Ile Leu
305                    310                    315                    320

ggg atc tca cat gaa gaa gta gac tga                                 987
Gly Ile Ser His Glu Glu Val Asp
                325
```

```
<210> 42
<211> 328
<212> PRT
<213> Mus musculus

<220>
<221> 107
<222> unsure
<223> Xaa = unknown amino acid

<400> 42
Met Cys Ser Thr Ser Val Tyr Asp Leu Glu Asp Ile Pro Leu Glu Asp
  1               5                  10                  15

Asp Asp Pro Asn Ser Ile Glu Phe Lys Ile Leu Ala Phe Tyr Ala Arg
            20                  25                  30

His His Val Phe Lys Asn Thr Pro Ala Val Phe Ser Pro Lys Leu Ser
```

```
              35                        40                        45
Arg Thr Arg Ser Leu Ser Gln Lys Ala Leu Gly Thr Trp Ser Thr Asp
    50                      55                      60

Ser Trp Thr Gln Val Ser Leu Pro Cys Arg Gly Ser Pro Ser Ser Glu
65                      70                      75                      80

Lys Asn Ile Ser Leu Gly Lys Lys Lys Ser Ser Trp Arg Thr Leu Phe
                85                      90                      95

Arg Val Ala Glu Lys Glu Glu Gly Leu Pro Xaa Ser Pro Lys Glu Ile
                100                     105                     110

Arg Ala Gln Gly Pro Gln Gly Pro Phe Pro Val Glu Arg Gln Ser Gly
                115                     120                     125

Phe His Asn Gln His Trp Pro Arg Ser Leu Ser Ser Val Glu Gln Pro
    130                     135                     140

Trp Arg Val Lys Leu Trp Ile Pro Lys Trp Leu Val Leu Pro Thr Glu
145                     150                     155                     160

Trp Leu Lys Leu Phe Thr Pro Gly His His Gln Met Ser Ser Thr Ala
                165                     170                     175

Arg Glu Glu Ala Ser Ser Lys Arg Gly Ser Arg Arg Phe Leu Tyr Phe
                180                     185                     190

Arg Phe Glu Gly Pro Trp Asp Ser Lys Asn Lys Asp Gly Glu Asp Gln
                195                     200                     205

Ile Ile Ser Lys Ile Val Glu Leu Leu Lys Ser Ser Gly Asp Gln Leu
    210                     215                     220

Gly Arg Glu Ile Lys Lys Asp Lys Ala Leu Met Ser Ser Phe Gln Asp
225                     230                     235                     240

Gly Leu Ser Tyr Ser Thr Phe Lys Thr Ile Thr Asp Leu Phe Leu Arg
                245                     250                     255

Asp Val Asp Thr Arg Gly Glu Ser Glu Val Lys Ala Arg Gly Phe Lys
                260                     265                     270

Ala Ala Leu Ala Ile Asp Ala Ile Ala Lys Leu Thr Ala Ser Asp Asn
                275                     280                     285

His Pro Met Asn Arg Met Leu Gly Phe Gly Thr Lys Tyr Leu Lys Glu
    290                     295                     300

Tyr Phe Ser Pro Trp Val Gln Gln Asn Gly Gly Trp Glu Lys Ile Leu
305                     310                     315                     320

Gly Ile Ser His Glu Glu Val Asp
                325
```

```
<210> 43
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 43
gggctcgaga tgtgcagcac cagtgtgtat g                            31


<210> 44
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
      primer

<400> 44
ccaagcttta agtctacttc ttcatgtgat atccc                        35
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